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THE DISTRIBUTION AND SIGNIFICANCE OF ORGANICALLY BOUND IODINE IN THE ASCIDIAN *CIONA INTESTINALIS* LINNAEUS

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(Plate I and Text-fig. 1)

INTRODUCTION AND PREVIOUS WORK

Attention has already been drawn (Barrington & Franchi, 1956*a*) to the presence of organically bound iodine in the endostyle of *Ciona*.[1] The purpose of the present work is to provide a fuller account of the distribution of this iodine, to examine how far the binding process is inhibited by the action of the goitrogen thiouracil, and to provide some cytological data for comparison with conditions in the endostyle of the ammocoete larva of the lamprey (Barrington & Franchi, 1956*b*). The importance of this latter aspect arises from recent demonstrations that significant amounts of thyroxin may be formed in various invertebrates (see, for example, Gorbman, Clements & O'Brien, 1954), possibly as the result of the iodination of skeletal scleroproteins. It has been plausibly suggested (Gorbman, 1955) that thyroid hormone may initially have arisen in this way as a biological accident; the thyroidal biosynthesis which is found in vertebrates, and which depends upon the secretion of thyroglobulin (Roche & Michel, 1955), would thus be a later evolutionary development consequent upon the iodination product having become metabolically important in the vertebrate line. From this point of view such protochordates as the ascidians occupy a position of key importance, and the crucial question to be answered is whether their iodine binding also is to be regarded, like that of invertebrates, as a chance by-product, or whether it shows any signs of being, like that of vertebrates, organized as a biochemical specialization.

MATERIAL AND METHODS

The animals were sent to Nottingham from the Laboratory of the Marine Biological Association, Plymouth. Some were fixed on arrival, while others were first immersed for 2 days in sea water containing 200 μc. of ^{131}I per litre. For investigation of the action of thiouracil the animals were immersed for

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[1] Some evidence for this has also been obtained in unpublished work of B. C. Abbott and D. A. McGill (see Report of the Council of the Marine Biological Association for 1953–4, this Journal, Vol. 33, p. 775).

1 day in sea water containing 0·03 g. of the goitrogen per litre, after which the radio-iodine was added and they were left for a further 2 days. Constant aeration was provided, and temperatures ranged from 9 to 15° C.

Material was fixed in Susa, mercuric-formol, Champy's fluid in sea water, Bouin's fluid in sea water, and Hollande's modification of Bouin's fluid without acetic acid. For staining and histo-chemistry use was made of the Azan technique, mucicarmine, Kull's technique, the periodic-acid-Schiff (PAS) procedure, the pyronin-methyl-green test for ribonucleic acid (RNA), the ferric ferricyanide test (Fisher, 1953), and fluorescence microscopy; some further reference to these methods will be found in Barrington & Franchi (1956*b*). Autoradiographs were prepared by the stripping-film technique of Pelc, as outlined by Pearse (1954), and were counterstained in Harris's haematoxylin and eosin.

OBSERVATIONS

THE ENDOSTYLE

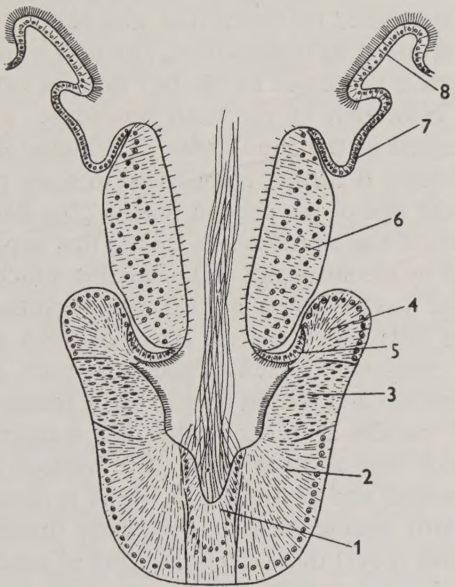
A comparative survey of the endostyle in seven species of ascidians has been reported by Sokólska (1931; see also Hûus, 1937), but the present study has disclosed some new points, both of fact and interpretation, which are of importance for the analysis of the functions and homology of the organ. In *Ciona* (Millar, 1953) it has the form of a deep groove, the epithelial wall of which is composed of a number of distinctive cell types; these will be referred to here for convenience as numbered zones (Text-fig. 1).

Zones 1–6

In the mid-ventral line, at the base of the groove, there is a group of cells (zone 1) which bear exceptionally long cilia. Near their nuclei there are occasional large vacuoles containing material which may be brownish in colour; the significance of these is obscure, but distally there is clear evidence of secretory function, for the apical part of the cell body contains material (diffuse or granular according to the mode of fixation) which stains intensely with aniline blue and with the PAS procedure, and which is the only product in the endostylar epithelium to react positively with mucicarmine. These cells differ, however, from the more typical secretory cells of the three pairs of glandular tracts (see below) in showing neither a large nucleolus nor a significant amount of RNA. Their cell bodies are slender and elongated, and their nuclei are crowded into two rather irregular layers, but the zone extends on each side as a layer of much shallower cells in which both the secretory contents and the ciliation are progressively reduced, the tips of these extensions becoming continuous with the ventral glandular tracts.

The latter are formed of elongated columnar cells (zone 2); each of these has a large spherical nucleus, a very conspicuous nucleolus, and cytoplasm rich in RNA, but it is difficult to judge whether they bear any cilia. In

Sokólska's figures these cells are shown lying more or less parallel with each other, and covered over (and therefore separated from the endostylar lumen) by an entirely separate layer of cubical cells. It is difficult to understand what could be the significance of such an arrangement, which would result in the separation of the glandular cells from the lumen into which they discharge, and in *Ciona* the actual situation proves to be a little different from this. The cells of the glandular tract are in fact arranged in a fan-shaped pattern (Text-fig. 1), with their apices crowded together in a narrow zone at which the discharge of their secretion must take place, and it is the lateral extension of the zone 1 cells over the main body of the fan which gives the misleading impression that the secretory cells are completely cut off from the lumen.



Text-fig. 1. Transverse section of the endostyle of *Ciona* (diagrammatic). The eight zones of the epithelium are indicated by corresponding numbers; for further explanation see text.

This detail is of some importance, not only because it makes the organization of the ventral tract functionally intelligible, but even more because it emphasizes the close resemblance between the endostyle of *Ciona* and that of the ammocoete larva. The cells of the glandular tracts in the latter animal are very like these zone 2 cells, and are arranged in a similar fan-shaped pattern, with discharge taking place through a narrow plug formed by the fusion of the tips of the cells.

The secretory functions of the endostyle of *Ciona* appear to be complex, and an analysis of the activity of the glandular tracts is outside the scope of the present work. It must suffice to say that a characteristic feature of the cells of the ventral and median tracts is the accumulation above the nucleus of a material, presumably a secretory product, which stains pale grey-blue with the Azan technique, gives a weak PAS-positive reaction, and is negative to mucicarmine. This is sometimes seen as a compact mass lying within a large vacuole, but its actual form is probably much influenced by artifact, and in some preparations, notably after Champy or Bouin-Hollande (without acetic) fixation, it is not sharply demarcated from the surrounding cytoplasm.

The ventral glandular tract is abruptly succeeded by zone 3, composed of slender ciliated cells which are closely crowded and which have their granular nuclei irregularly arranged in a number of layers. RNA is negligible in these cells, but the presence of granular inclusions, PAS-positive after fixation in Bouin-Hollande (without acetic), suggests that they may have some secretory function. They are succeeded by the median glandular tract (zone 4), which resembles the ventral one in its organization, although some differentiation in the secretory products is suggested by the fact that the cytoplasm of the two tracts differs in its coloration by the Azan procedure; a somewhat similar situation has been noted in the ammocoete (Barrington & Franchi, 1956*b*). Resemblance to the latter is further emphasized in some preparations by the apparent fusion of the tips of the cells to form a plug-like structure exactly comparable with that of the ammocoete's tracts; this appearance may, however, be exaggerated by the shrinkage and distortion which is clearly induced by fixation. The zone 5 cells form a short length of epithelium composed of more or less cubical cells which contain traces of RNA and a few granular inclusions. Zone 6 comprises the dorsal glandular tract, which differs in its structure from the median and ventral ones in being formed of elongated cells which are arranged parallel with each other like a more normal columnar epithelium, and which bear scattered cilia. The nucleoli are large, although less so than in the median and ventral tracts, RNA is plentiful, and secretory material (staining with aniline blue) is irregularly distributed in vacuoles. According to Sokólska (1931) three different types of product are discharged from these cells, and it is certainly clear, as she remarks, that the so-called mucous secretions of the ascidian endostyle must be a product of considerable complexity.

In a recent account of the ammocoete's endostyle (Barrington & Franchi, 1956*b*) a distinction has been made between alimentary and thyroidal functions. From this point of view, which seems also applicable to *Ciona*, the portions of the endostyle so far described, constituting the main bulk of the organ, must be regarded as primarily alimentary; there is probably more to be learned as to the full function of its secretions, but they are well known in general terms to be concerned with the trapping of food particles (Orton, 1913). Over the whole of this region (zones 1–6) bound iodine is inconspicuous in autoradiographs and is possibly absent altogether except in association with the long cilia of zone 1, where a small amount of iodine is commonly to be found; this, however, has a separate significance which will be discussed below (p. 8). In some areas of the epithelium of some specimens, however, the density of the autograph seems to be slightly greater than that of the background, particularly in zone 1 and in the upper part of zone 6, and it is just possible that this is indicative of a slight capacity for iodine binding; it is certain, however, that this would attract little attention if it were not for the highly significant images obtained from the remainder of the organ (zones 7 and 8).

Zone 7

Dealing first with zone 7 (Text-fig. 1; Pl. I, fig. 5), iodine is here found distributed more or less evenly over the cell bodies throughout the whole extent of this epithelium; the autoradiographic image (Pl. I, figs. 6, 11) is not dense, but it is a very definite and constant feature of this zone, and it seems evident that iodine binding must be occurring in the cells. Its clear definition in this particular zone shows that this binding cannot be merely a chance by-product of the general secretory activity of the endostyle, and a further examination of the properties of zone 7 provides good evidence that a specialized secretory process is, in fact, involved, for its cells contain numerous granules of variable size, and may sometimes show a slight indication of RNA. Often there appears to be one large granule per cell (Pl. I, fig. 12), but in some preparations additional smaller ones can be seen, and it is probable that a fusion of a group of these into larger ones may occur as an artifact of fixation. Mucus-like material, not dissimilar in shape although with less sharply defined contours, is plentiful elsewhere in the pharyngeal epithelium, so that the properties of the zone 7 granules, in comparison with the inclusions of other cells, need careful examination; they may be summarized as follows:

(*a*) Colour. They are pale yellow-brown in colour, a property not found anywhere else in the endostylar or pharyngeal epithelium except in the material seen in vacuoles in some of the zone 1 cells (see above, p. 2). The pigment, both in the latter and in the zone 7 granules, is bleached after 24–48 h treatment with 10% hydrogen peroxide, and is thus possibly melanic, although the initial colour is sometimes so weak that this result may not be of much significance.

(*b*) Fluorescence. After fixation in Susa, mercuric formol, or Bouin in sea water the granules exhibit a yellow-brown fluorescence which becomes very pale yellow after bleaching with hydrogen peroxide. Although fluorescence is a conspicuous feature of cells in the blood sinuses, it is found nowhere else in the endostylar or pharyngeal epithelium with the fixatives mentioned; in particular, the brown material in zone 1 is not fluorescent. After fixation in Bouin-Hollande (without acetic) some finely particulate whitish fluorescence appears in zone 6 and in the mucous region of zone 1, but the fluorescence of zone 7, although less striking than with the other fixatives, remains distinctive.

(*c*) Azan staining. The zone 7 granules commonly stain a characteristic bright orange; the inclusions elsewhere show a varying degree of affinity for aniline blue, the apical secretion of zone 1 and the secretion of the pharyngeal epithelium staining deeply with this.

(*d*) PAS reaction. The granules give a moderately strong positive response. A noticeably stronger response is given by the secretion of zone 1 and of the pharyngeal epithelium, while the response of the remaining secretory products of the endostyle, together with the brown material in zone 1, is weak and indefinite.

(*e*) Mucicarmine. The granules are negative to mucicarmine, as also are all of the products of the endostyle, with the exception of the zone 1 secretion (see above). An occasional indefinite response may be given by the pharyngeal epithelium, but this also is commonly negative.

(*f*) Ferric ferricyanide test. The granules give a moderate but quite definite positive response. The secretion of the pharyngeal epithelium is also positive, but more strongly so, giving a deep blue colour. A strong response is also obtained from some of the contents of zone 1; the response of the remainder of the endostyle is indefinite, although the cytoplasm of the glandular cells may respond to a moderate extent. After fixation in Bouin-Hollande (without acetic) the response of the zone 7 granules is relatively more intense.

The above tests are not, of course, specific, a positive ferric ferricyanide response, for example, indicating no more than the presence of reducing groups (Adams, 1956), and the results are clearly influenced to some extent by the nature of the fixative. It can, however, be concluded that there is present in zone 7 a particular type of secretory inclusion which displays characteristics not found in the same combination elsewhere in the endostylar or pharyngeal epithelium. No useful inferences can be drawn at this stage as to the distinctive chemical nature of this secretion, but it can be said that the granules are associated with iodine binding, and it is significant that their properties, as outlined above, are virtually identical with those of the secretion of the thyroidal epithelium of the endostyle of the ammocoete (Barrington & Franchi, 1956*b*). It seems reasonable, therefore, to conclude that they are homologous with the latter, and, through them, with thyroid colloid, and that they thus constitute evidence for the existence in *Ciona* of a specialized secretory activity providing the molecular basis for the iodination process. On this interpretation the latter is not, therefore, a chance by-product of endostylar secretion, but seems very likely to represent a truly thyroidal biosynthesis. For this reason the zone 7 granules will be referred to as *thyroidal granules*, the name already applied to the corresponding secretion of the ammocoete.

Zone 8

In certain sections one or more of the thyroidal granules of zone 7 can be seen lying in the endostylar lumen, or else at the tip of a cell as though extrusion were about to take place. Such appearances could, however, very well be an artifact of fixation or of sectioning, and they are too infrequent to be accepted as reliable evidence of discharge. On the other hand, there is reason for believing that in the ammocoete the iodinated product may be extruded into the endostylar lumen in a more fluid and non-staining form, and it is possible that the same may apply to *Ciona*. The importance of this matter lies in its relevance to the interpretation of the condition of the zone 8 epithelium (Text-fig. 1). This is somewhat thicker than zone 7, and is composed of strongly ciliated cells which lack RNA and which show less sign of secretory activity than any other part of the endostylar epithelium. These cells are known (Orton, 1913) to be responsible for driving the secretion of the endostyle laterally out of the groove and on to the pharyngeal wall, up which it is then swept by the cilia of the latter (see below, p. 8). It follows that any iodinated product which may be discharged from zone 7 would also be swept over these zone 8 cilia and some might well be adsorbed to them; thus would arise autoradiographic images which would, however, indicate a transmission of bound iodine across the cells rather than the existence of it within them.

This appears to be the most likely explanation of the fact that zone 8 actually gives a much more intense image than does zone 7 (Pl. I, figs. 5, 6), for close inspection of the distribution of the iodine shows a marked difference between the two zones, the weaker image of zone 7 being almost uniformly distributed over the cells, while that of zone 8 shows an intense concentration over the cilia and cell borders. Pl. I, figs. 6 and 11, illustrate this feature, although it is much less clearly defined in the photomicrographs than in the original preparations. The iodine image extends also over the cell bodies, but this extension is much less intense than that of the surface concentrations, and could quite well be a result of random scatter from them. There is, too, the possibility that if an iodinated secretion does accumulate over the surface of the epithelium there might be some diffusion from it into the cytoplasm. However, it is clearly impossible to be sure that no binding of iodine takes place within the zone 8 cells, but the absence of any obvious secretory basis for it suggests that its occurrence is highly improbable. Moreover, the view that the iodine image over zone 8 is a consequence of the movement of iodinated secretion by the cilia is strongly supported by consideration of the distribution of bound iodine elsewhere in the pharynx, as will now be explained.

THE PHARYNGEAL CONTENTS

First, there is clear evidence that an iodinated product is mixed with the visible secretion of the endostyle and pharynx, for the food cords in the lumen are found to be strongly iodinated (Pl. I, figs. 1, 2). Here, however, a complication is admittedly introduced by the capacity of marine organisms to accumulate bound iodine (see above, p. 1), for any such organisms included in the food would be expected to show this same property. This was, in fact, strikingly exemplified by one specimen in which a large unidentified arthropod within the pharynx showed a substantial concentration of bound iodine within its exoskeleton (Pl. I, figs. 1, 2). This property could hardly, however, account for all of the iodination of the food cords, the exogenous contents of which are very variable in amount and miscellaneous in nature, including *Ciona* sperm, diatoms and unidentifiable cellular material and detritus. It is perhaps impossible to establish with absolute certainty that an iodinated secretion is added to the food cords from the endostyle, but the strong probability that this does happen is indicated by the fact that radioactive secretion, with very little exogenous material in it, can be observed in the mouth of the organ (Pl. I, figs. 5, 6). Equally significant is the almost invariable association of a weak but definite iodine image with the long cilia which arise from zone 1, for these are thought to have no function in the transporting of food particles, but are believed to assist in the deflecting of the endostylar secretion on to the cilia of zone 8 (Orton, 1913).

THE PHARYNGEAL EPITHELIUM

A second significant aspect of iodine distribution within the pharynx arises from the complicated structure of the wall of this organ. A current of water is maintained by the cilia of the gill openings or stigmata, but internally to the latter there is a system of longitudinal bars from which papillae project into the pharyngeal cavity (Roule, 1884; Berrill, 1950). Now the long threads of secretion which trap the food particles are swept up the walls mainly by the cilia of these papillae, aided in part by a waving movement of the bars themselves (Orton, 1913), with the ciliated dorsal languets becoming involved in the backward movement of the main food cord. If, therefore, there is any tendency for the iodinated component of the endostylar secretion to accumulate over the surfaces of the ciliated epithelia which propel the latter, as has been suggested above for zone 8, an autoradiographic image should be conspicuous on the ciliated regions of the papillae and languets. This is, in fact, readily seen to be so (Pl. I, figs. 1, 2, 9 and 10), the image, as with that of zone 8, showing a concentration over the surface rather than a uniform distribution over the cell bodies. In sharp contrast to this, the ciliated epithelia of the stigmata, which, by virtue of their position, are largely removed from contact with the endostylar secretion, yield very much lighter and often quite negligible images.

THE INTESTINAL CONTENTS

Within the intestine the food cords are distinguishable into two components, one being material which has entered from the pharynx and which is recognizable by the variety of its contents, and the other a clear mucus-like substance, positive to mucicarmine, which has no food material mixed with it, and which is a secretion of the mucus cells of the intestinal epithelium. Autoradiographs demonstrate an important difference between these two components (Pl. I, figs. 7, 8), for the former gives an iodine image while the latter does not; here, then, there is no evidence for any association of iodine binding with the normal process of alimentary secretion.

THE TEST

The presence of iodine in the test of ascidians has long been known from the work of Cameron (1914, 1915), who, without reporting on its chemical form, noted that it was especially abundant in the surface layer. The test is now known to be composed of two parts, the tunic proper and the superficial cuticle. The former is made up of the cellulose-like tunicin, with which is associated some glycoprotein, while the cuticle consists of pure protein and is thus chemically different from the remainder (Pérès, 1948). This difference is emphasized by the mode of development, for while the epidermis is mainly responsible for secreting the basic substance of the test, the cuticle arises from 'Tropfenzelle'; the latter initially contain a glycoprotein complex, but after migrating into the test they appear to give up the carbohydrate component to the tunicin and the remainder of their content is subsequently incorporated into the cuticle.

In view of these facts it is of interest that autoradiographs clearly demonstrate (Pl. I, figs. 3, 4) an accumulation of bound iodine in the cuticle, while none appears to be present in the remainder of the test. Mention has been made above (p. 1) of the way in which bound iodine becomes incorporated into the scleroproteins of the skeletal structures of invertebrates; the nature of the proteins of the test clearly demands further investigation from this point of view, for if the tough outer cuticle could be shown to contain scleroprotein the presence in it of bound iodine would be readily explained as a similar by-product of skeletal secretion. The facts at present available certainly suggest that iodination in the test is a process quite distinct from that in the endostyle, although it is tempting to speculate that the existence of the former might have been the starting point for the evolution in ascidians of a biochemically useful iodinated product.

In the earlier stages of this work there appeared to be indications of the association of bound iodine with the mantle epithelium (Barrington & Franchi, 1956*a*), but further studies have not substantiated this, and at the present time significant amounts of bound iodine have only been found consistently in the regions mentioned above. The iodination of the test merits further investigation, however, for, as has been suggested here, it raises an issue of some evolutionary interest, and it is hoped to report on it further in relation to the mode of secretion and regeneration of this tissue. Moreover, the existence of bound iodine in the stolonic septum of *Perophora* (Gorbman, 1941) shows that the present description does not exhaust the possibilities of iodine binding in ascidians.

THE ACTION OF THIOURACIL

In considering the effects of thiouracil it is necessary to bear in mind its supposed mode of action. Thyroidal biosynthesis is thought to depend upon the iodination and subsequent condensation of the tyrosine residues in thyroglobulin, the specific protein of the thyroid gland (Roche & Michel, 1955), but the means by which chemical goitrogens produce their interference with this process are not fully understood. It seems likely, however, that thiouracil, acting perhaps as a reducing agent, prevents the liberation from iodide of free iodine, the presence of the latter being essential if the iodination of tyrosine is to take place. This requirement has been mainly studied in relation to thyroidal biosynthesis, but it appears to be a necessary condition for any protein iodination, including *in vitro* reactions (Rawson, Rall & Sonenberg, 1955). It would seem to follow from this that the iodination of skeletal scleroproteins by radioactive iodide must depend upon the release of free iodine, and that this in its turn might well depend, at least in part, upon an appropriate oxidizing enzyme system which might, therefore, be inhibited by thiouracil. This means that a demonstration of such inhibitory action in any tissue cannot be held as a proof that it is carrying on thyroidal function in the strict sense of the iodination of a specific thyroid protein.

The effect of thiouracil treatment upon *Ciona* is to eliminate almost completely all traces of bound iodine from the sites mentioned above. None at all can be detected in the endostylar epithelium or on the pharyngeal cilia. It is, however, sometimes possible to detect a very slight but significant amount in the food cords, both in the pharynx and in the intestine, but not, of course, in the purely intestinal mucus in the latter (see above, p. 9). It is not clear whether this iodine is associated solely with the exogenous food material, or whether it results from a small amount of bound iodine continuing to be discharged from the endostyle without the prior storage which would be needed to enable that organ to produce a significant autoradiograph, but it is of interest that a similar situation appears to exist in *Amphioxus* (Thomas, 1956), the association of iodine with food particles being demonstrable after thiouracil treatment has eliminated it from the endostyle of that animal. The amount involved in *Ciona*, however, is very much less than in control specimens and could easily be overlooked if it were not being specially sought, and it is clear that the goitrogen substantially arrests the normal iodination process. This applies also to the cuticle of the tunic, for in thiouracil-treated specimens the bound iodine is either completely lacking or is present in a greatly reduced amount. It is impossible to judge the full significance of these results until there has been further clarification of the biochemical basis of the iodination process in the endostyle and cuticle, and until the mode of action of thiouracil is better understood. For the reasons already stated, however, the behaviour of the cuticle cannot be taken as evidence that this region is concerned with specifically thyroidal biosynthesis.

DISCUSSION

The observations recorded here show a close resemblance between the endostyle of *Ciona* and that of the ammocoete, not only in the organization of certain of the glandular tracts, but more especially in the ability of parts of the epithelium to bind iodine, and in the association of the latter property with a characteristic secretory product, here called the 'thyroidal granule'. So close is this resemblance that there can now be little doubt as to the correctness of the classical view that the protochordate endostyle is homologous with that of the ammocoete and, through the latter organ, with the thyroid gland of the vertebrates. It is necessary to consider further, however, the justification for regarding iodine binding in the ascidian endostyle as a truly thyroidal biosynthesis.

Spaul (1928) was unable to accelerate metamorphosis in frog tadpoles by feeding them with *Ciona* endostyle, but in view of the relative weakness of the autoradiograph of the latter organ as compared with that obtained from mammalian thyroid it may be doubted whether the amount of tissue used by him would have been adequate for securing a positive result. As against this negative finding, Sembrat (1953) was able to bring about metamorphic changes in axolotls by inserting into them as many as sixty-five dried endostyles of *Amphioxus*, in which organ iodine binding is known to occur (Thomas, 1956), and in view of this, and of the known occurrence of thyroxin in the ammocoete endostyle (Leloup & Berg, 1954), there is clearly no *a priori* reason why thyroid hormone should not be present in the endostyle of *Ciona*. It is hoped to report later on the chemical form of the bound iodine of this organ and of the test, but in the meantime it must be said that, for reasons indicated above (p. 1), this particular issue now seems less crucial than the question as to whether or not the iodine binding is organized as a biochemical specialization.

It is for future work to show whether, as seems quite likely, the iodination of the cuticle in *Ciona* is a chance result of the presence there of scleroproteins. The situation in the endostyle, however, seems much better defined, for the present work has shown that iodination in that organ takes place within a narrowly limited zone which is cytologically specialized in a manner directly comparable with the thyroidally active regions of the ammocoete's endostyle. The inference is that the product of iodination is a thyroid hormone which has become metabolically important for the ascidian, and that a particular epithelium is set apart for secreting, as thyroidal granules, the necessary molecular basis for the iodination process. It is not possible as yet to suggest what might be the functional importance of this hormone, but it is clearly essential that this inference should now be tested by examining whether thyroxin can be shown to exert any specific effects upon ascidians. At this stage also it is not clear whether any significance is to be attached to the slight traces of bound iodine in other parts of the endostyle. Such traces might represent a generalized capacity from which the special property of zone 7 evolved, but study of a wider range of species is necessary before any useful opinion can be expressed on this.

The autoradiographs do not disclose the route by which the iodine reaches these cells; presumably iodide might be absorbed by them direct from the water, or it might reach them in the body fluid after absorption elsewhere (by, for example, the pharyngeal wall). In this connexion it seems worth noting that the zone 7 cells are separated from the underlying body fluid by a rather thick layer of connective tissue; no doubt this would not preclude absorption through the latter, but, having regard also to the situation of this zone at the upper edge of the endostylar groove where it must be bathed by moving sea water, circumstances would seem to favour the possibility of direct absorption of iodide from the pharyngeal lumen. This would, of course, partly explain the use of endostylar epithelium for thyroidal biosynthesis, and other advantages are its well-developed secretory capacity, which would provide a basis for the evolution of the thyroidal granules, and the ease with which the hormonal product can be mixed with the remainder of the endostylar secretions and so be conveyed into the intestine. From there it is presumably taken up into the body tissues (although there is at present no proof of this), and it is the development of this particular route of absorption which, as Thomas (1956) has also pointed out in connexion with his study of *Amphioxus*, explains why thyroid hormone can be successfully administered by mouth throughout the vertebrates.

Comparison with the ammocoete is complicated by the very different organization of the pharynx in the vertebrates, but it is clear that the division of the endostyle in the latter animal into two halves, amounting to its virtual duplication, and its posterior elaboration into a spiral coil, provide a very large surface area within the limited space available. The proportion of this area devoted to iodine binding is, moreover, very substantially greater than in the ascidian, while many of the thyroidally active cells are columnar in form and, with their large complement of thyroidal granules, seem much more specialized than the corresponding cells of zone 7 (Barrington & Franchi, 1956*b*). These facts suggest a more intensive development of iodine binding in the ammocoete, and this must surely be correlated with the diminished availability of iodine which would have been an inevitable consequence of the migration into fresh water which marked the evolution of vertebrates from the protochordates. It may be suggested also that the form of the endostyle in the ammocoete, where it appears as a sac with only a narrow opening, is not as well adapted as is an open groove for the uptake of iodide from the water circulating through the pharynx; since, therefore, the endostylar epithelium in this animal has close contact with well-developed blood capillaries it seems probable that uptake from the blood stream may have become established with this advance in specialization, although here again the autoradiographs offer no clear evidence.

It is of great interest that iodine binding has also been shown to occur in the endostyle of *Amphioxus* (Thomas, 1956), for this establishes the phenomenon as a common property of the organ in all three of the groups (Tunicata, Cephalochordata and Vertebrata) in which the latter is present. The endostyle of *Amphioxus* has only two pairs of glandular tracts, and the iodine binding appears to be located in or near the lateral pair, a fact which has led Thomas to regard the endostylar mucus as the direct evolutionary forerunner of thyroid colloid. The present work shows, however, that there are specialized iodine binding cells in the ascidian endostyle which are distinct from the glandular tracts and which would seem to resemble the thyroidally active cells of the ammocoete much more closely than do the mucus cells of *Amphioxus*, despite the fact that the latter animal, with its well-developed metamerism, is in some respects much closer to the vertebrates than are the ascidians. This situation, at first sight somewhat paradoxical, clearly needs further investigation. Its explanation may, however, be found in some recent arguments of Berrill (1955), who has developed with great cogency the view that *Ciona* represents a truly primitive level of chordate organization from which the vertebrates could have been derived by the process of neoteny on which Garstang (1929) placed such emphasis. Berrill suggests that *Amphioxus* is a specialized offshoot of the main chordate stem, and represents a secondary return to a fully marine existence from a stage at which the early chordates were already ascending estuaries and becoming established in fresh water. He ascribes to this the secondary degeneration of their anterior sensory equipment, and it would seem logical to expect that a comparable degeneration in the iodine-binding mechanism would result from such a return to an iodine-rich habitat. For the present this suggestion must remain a matter for speculation, but in the meantime some support for such an interpretation is possibly to be drawn from Thomas's interesting observation that *Amphioxus* does not take up radio-iodine when it is first removed from its natural habitat, but begins to do so when it is kept in the laboratory in circulating water in which the iodine content is probably lower than in the outside sea water.

I am indebted to Mr T. Berbank for preparing the photomicrographs and to Miss J. M. Plumtree for technical assistance. Comparisons with the ammocoete larva have been rendered possible by work carried out on that animal in collaboration with me by Mr L. L. Franchi.

SUMMARY

Some features of the organization of the endostyle of *Ciona intestinalis* are described. Autoradiographs show that bound iodine is present in a limited area of the epithelium (zone 7), where it is associated with characteristic secretory inclusions which are found only in this zone. These are regarded as providing the molecular basis for the iodination process, and are termed 'thyroidal granules', since they resemble in certain properties the similarly named granules of the endostyle of the ammocoete; it is concluded that they are homologous with these and, through them, with thyroid colloid. Their existence provides evidence that the iodination process is a product of biochemical specialization, and implies that it results in the formation of an hormonal secretion. It is concluded from the distribution of bound iodine over the pharyngeal epithelium and in the food cords that this iodinated secretion is carried in the latter into the intestine. Bound iodine is also present in the cuticle of the test; it is suggested that this may differ from the situation in the endostyle in being a consequence of the iodination of a skeletal protein secretion such as is known to occur in many invertebrates. Prior immersion of the animals in thiouracil solution largely eliminates bound radio-iodine from all of the sites mentioned, although a little may sometimes be detectable in the food cords and cuticle. The results are discussed in the light of recent work on *Amphioxus* and the ammocoete larva, and attention is drawn to their bearing on current views on the relationship between vertebrates and protochordates.

REFERENCES

Adams, C. W. M., 1956. A stricter interpretation of the ferric ferricyanide reaction. *J. Histochem. Cytochem.*, Vol. 4, pp. 23–35.

Barrington, E. J. W. & Franchi, L. L., 1956*a*. Organic binding of iodine in the endostyle of *Ciona intestinalis*. *Nature, Lond.*, Vol. 177, p. 432

—— —— 1956*b*. Some cytological characteristics of thyroidal function in the endostyle of the ammocoete larva. *Quart. J. micr. Sci.*, Vol. 97, pp. 393–410.

Berrill, N. J., 1950. *The Tunicata*. London: Ray Society.

—— 1955. *The Origin of Vertebrates*. Oxford: The Clarendon Press.

Cameron, A. T., 1914. The distribution of iodine in plant and animal tissue. *J. biol. Chem.*, Vol. 18, 335–80.

—— 1915. The distribution of iodine in plant and animal tissue. *J. biol. Chem.*, Vol. 23, pp. 1–39.

Fisher, E. R., 1953. Observations on thyroid colloid. *Arch. Path.*, Vol. 56, pp. 275–85.

Garstang, W., 1929. The morphology of the Tunicata, and its bearing on the phylogeny of the Chordata. *Quart. J. micr. Sci.*, Vol. 72, pp. 51–187.

Gorbman, A., 1941. Identity of an iodine-storing tissue in an ascidian. *Science*, Vol. 94, p. 192.

—— 1955. Some aspects of the comparative biochemistry of iodine utilization and the evolution of thyroidal function. *Physiol. Rev.*, Vol. 35, pp. 336–46.

Gorbman, A., Clements, M. & O'Brien, R., 1954. Utilization of radio-iodine by invertebrates, with special study of several annelids and mollusca. *J. exp. Zool.*, Vol. 127, pp. 75–92.

Hŭus, J., 1937. Tunicata: Ascidiaceae. *Handb. Zool. Berl.*, Bd. 5, Hlf. 2, pp. 545–672.

Leloup, J. & Berg, O., 1954. Sur la présence d'acides amino-iodes dans l'endostyle de l'Ammocoete. *C.R. Acad. Sci., Paris*, T. 238, p. 1069.

Millar, R. H., 1953. *Ciona*. L.M.B.C. Memoir No. 35. (Liverpool: University Press).

Orton, J. H., 1913. The ciliary mechanisms of the gill and the mode of feeding in *Amphioxus*, ascidians and *Solenomya togata*. *J. mar. biol. Ass. U.K.*, Vol. 10, pp. 19–49.

Pearse, A. G. E., 1954. *Histochemistry, theoretical and applied*. London: Churchill.

Pérès, J. M., 1948. Recherches sur la genèse et la régénération de la tunique chez *Clavelina lepadiformis* Müller. *Arch. Anat. micr. Morph. exp.*, T. 37, pp. 230–60.

RAWSON, R. W., RALL, J. E. & SONENBERG, M., 1955. The chemistry and physiology of the thyroid. *The Hormones* (ed. Pincus & Thimann), Vol. 3, pp. 434–519.

ROCHE, J. & MICHEL, R., 1955. Nature, biosynthesis and metabolism of thyroid hormones. *Physiol. Rev.*, Vol. 35, pp. 583–610.

ROULE, L., 1884. Recherches sur les ascidies simples des côtes de Provence. *Ann. Mus. Hist. nat. Marseille*, T. 2, pp. 1–270.

SEMBRAT, K., 1953. Effect of the endostyle of the lancelet (*Branchiostoma lanceolatum* Pall.) on the metamorphosis of axolotl. *Zool. Polon.*, Vol. 6, pp. 3–19.

SOKÓLSKA, J., 1931. Contribution à l'histologie de l'endostyle des ascides. *Folia Morph., Warsz.* T. 3, pp. 25–34.

SPAUL, E. A., 1928. Comparative studies of accelerated amphibian metamorphosis. *J. exp. Biol.*, Vol. 5, pp. 212–32.

THOMAS, I. M., 1956. The accumulation of radioactive iodine by *Amphioxus*. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 203–10.

EXPLANATION OF PLATE I

Fig. 1. Scene in the pharynx of *Ciona*, showing, in transverse section, part of an unidentified arthropod to the left, a food cord to the right, and dorsal languets above. Azan.

Fig. 2. Autoradiograph of an adjacent section. Iodine is present in the exoskeleton of the arthropod, in the food cord, and over the ciliated lateral borders of the dorsal languets (see also figs. 9 and 10).

Fig. 3. Section through part of a colony of *Botryllus* which is growing upon the surface of the test of a *Ciona*. The cuticle of the former is at the top left and that of the latter at the bottom right. PAS.

Fig. 4. Autoradiograph of an adjacent section. Iodine is conspicuously concentrated in the cuticle of *Ciona* (extending across the bottom of the photograph) and is also present to a less extent in that of *Botryllus* (top left).

Fig. 5. Upper part of the right side of the endostyle of *Ciona* (compare Text-fig. 1). The upper part of zone 6 is at the bottom right, zone 7 is above it, and zone 8 forms a crescent to the left; the pharyngeal epithelium extends from the latter upwards and to the right. A small mass of secretion lies in the mouth of the endostyle towards the bottom left corner. PAS.

Fig. 6. Autoradiograph of an adjacent section. Iodine is present over zones 7 and 8, and in the small mass of secretion at the bottom left-hand corner. At the top of the crescent formed by zone 8 there is some indication of the concentration of iodine at the surface of this epithelium (see text).

Fig. 7. Food cord in the intestine of *Ciona*. The dense pharyngeal component, with exogenous material, extends to the left, and is sharply distinguished from the clear intestinal secretion which lies to the right. Azan.

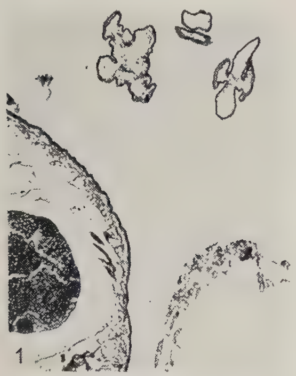
Fig. 8. Autoradiograph of an adjacent section. Iodine is conspicuous in the pharyngeal component and is absent from the intestinal secretion.

Fig. 9. Lateral border of a dorsal languet; a small amount of secretion overlies the columnar cells, and is associated with their cilia. PAS.

Fig. 10. Autoradiograph of an adjacent section. Iodine is concentrated over the surface of the ciliated columnar epithelium (see text), but is absent from the unciliated cubical epithelium below. The dense image at the top centre is associated with secretion overlying the cells (compare fig. 9, in which some of this secretion is visible).

Fig. 11. Autoradiograph of zone 7 (below) and zone 8 (in the upper third of the photograph). Iodine is equally distributed over the former, but is concentrated at the surface of the latter (see text, and compare figs. 6 and 10).

Fig. 12. Zone 7 epithelium. Thyroidal granules are in focus at intervals; see particularly the large dark granule in the lighter area where the epithelium is bending to the left in the upper part of the photograph. PAS.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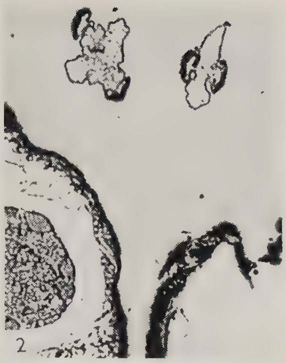
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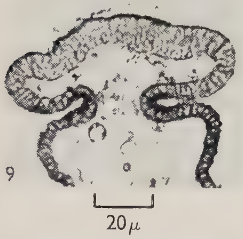
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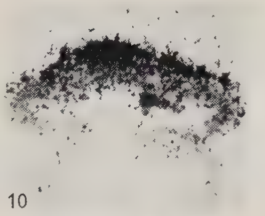
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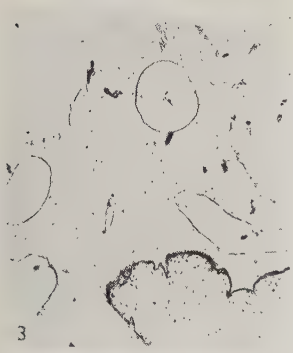
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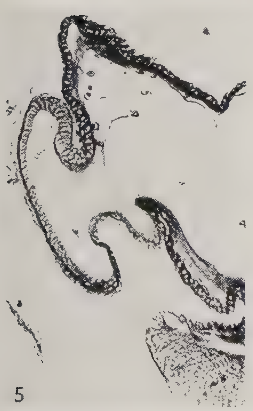
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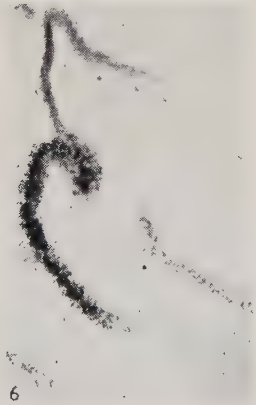
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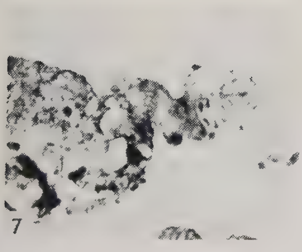
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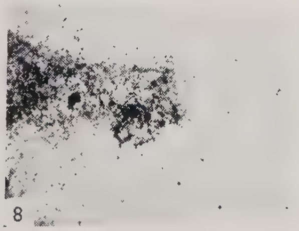
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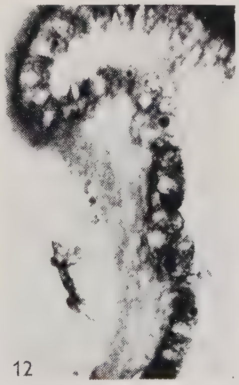
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A NEW QUANTITATIVE PLANKTON NET

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(Text-figs. 1–6)

INTRODUCTION

In the study of the production of organic matter in the ocean, it is necessary to find out not only how quickly organic matter is produced, but also how the material is distributed in the various stages of the organic cycle.

Ignoring for the moment difficulties created by the patchiness of plankton distribution, it is possible by taking a suitably sized sample of sea water to obtain a fairly representative sample of small organisms such as the unicellular algae. With the larger organisms constituting the zooplankton, the problem of sampling becomes more complex. Very few of the planktonic animals are small enough or abundant enough to be sampled adequately by a sample of water, and it is necessary to resort to the use of nets, filtering a large quantity of water, to obtain sufficient numbers of organisms. Further complications are introduced by the immense size range of the zooplankton organisms, some of the smaller ones being even smaller than some of the larger phytoplankton, while there is also the fact that some of the larger ones are apparently quite capable of evading capture by their greater powers of swimming.

The present work was aimed at studying the distribution and fluctuations of the biomass of the zooplankton. Although it was realized that no one sampling device could meet all the difficulties involved and give an accurate picture of the zooplankton as a whole, it was hoped that some single means might be found for sampling the bulk organisms of the zooplankton, and thereby collect material from which some valid inferences might be drawn with minor reservations as to the more active forms.

Furthermore, it was hoped to extend this study into both polar and tropical waters, and this also impinged on the method of sampling adopted. It is a well-known fact that organisms filling the same ecological niches are generally of much larger size in cold polar waters than in warmer waters. As an illustration of this, in temperate surface waters an averaged size Chaetognath, say *Sagitta enflata*, may reach a length of about 20 mm, whereas in the Antarctic the size of a similar creature, *Sagitta gazellae*, may be about 80–90 mm. This point was rather important if the studies made in these different regions were to be comparable.

As most of the work was designed to take place in oceanic waters, another requirement was some form of closing device to enable the different water layers to be sampled.

The final and principal requirement for the investigation was to be able to relate the catch of the net to the actual amount of water from which it was filtered.

It was evident that no existing net could fulfil all of these requirements. The Clarke–Bumpus net (Clarke & Bumpus, 1950) was probably the nearest answer, but it was obviously too small to cope with the larger organisms, and also suffered the disadvantage of being designed for oblique or horizontal hauls. The practical difficulties of using an oblique net in deep water are rather great, especially as its operation requires so much time. Moreover, a vertical haul has the special advantage that it can conveniently be dovetailed with other work. So far as we are aware no suitable vertical net was in existence to satisfy our requirements, and it appeared to us, therefore, that a new piece of equipment would have to be designed.

From the results of expeditionary work in the past, it is apparent that the vertically hauled Nansen net (Künne, 1929) is a remarkably good sampling device. As it is only 70 cm diameter at the mouth, it obviously cannot be expected to sample such large and active organisms as *Euphausia superba* but, nevertheless, towed at a speed of 1 m/sec (2 knots) it is an adequate sampler of the normal bulk organisms of the zooplankton. Furthermore, the net has been used extensively in various parts of the world, and seems to produce reasonably comparable samples from a wide variety of regions.

The main disadvantages of the Nansen net are first, that there is no device to determine how much water it filters and, secondly, even if this were known, the mesh of the net is of two different sizes, namely 40 meshes per linear inch in the forepart of the net and 74 m.p.i. in the after part, and it would be impossible to tell how much water went through each mesh section.

The obvious course seemed to be to adapt the Nansen net so that it would be suitable for quantitative work, but it was clearly advantageous to retain in any altered version as many as possible of the desirable features of the Nansen net.

The first question of mesh size had to be settled experimentally. First, the front French netting section was replaced with canvas and then in consideration of Wiborg's (1948) findings relating to the advantages of certain meshes, various meshes were tried in the filtering part of the net. Eventually a size of 74 m.p.i. throughout was chosen as being the the best compromise between filtering capacity and smallness of organisms taken. The size of aperture of this silk is similar to that of no. 8 standard grade Dufour bolting silk, and in the dry silk averages 200 μ while in the wet silk it shrinks slightly to between 160 and 180 μ.

The next, and most important point, was the measurement of water flow through the net. This was particularly desirable for the deeper hauls where there was no indication of the angle at which the net may be hauled up through the different water layers (that is when the drift of the ship causes the wire to stray from the vertical). It was intended that the net would be closed by the Nansen (1915) method, and so it was also desirable that the actual depth of closure might be known. It was evident that to suit the purpose, no available flowmeter existed. What was really required was a meter capable of showing the performance of the net and depth at which it fished. In other words, a meter which could give a graph of the amount of water filtered plotted against depth. The advantages of such a device would be enormous. Any clogging of the net by superabundance of plankton would at once be apparent and the depth at which it occurred would be known. The angle of hauling of the net would not matter since the actual volume filtered between two levels could be determined, and also any irregularity of performance of the net would be obvious.

Even if such a meter were designed, how was it to be fitted in the net? On hydrodynamic considerations it appeared most reasonable to measure the water after it had come through the net. To this end some trials were made. A net was enveloped in a cylindrical canvas sleeve and the meter placed behind the net. This apparatus proved quite unmanageable, as it sank so slowly through the water. The idea had to be abandoned and the meter was then mounted in the mouth of the net where it functioned excellently. The closing problem was later overcome by mounting the meter in a brass drum between a front canvas sleeve and the silk filtering part of the net. The net could then be closed in the normal manner by the Nansen method, in front of the meter.

Some 160 hauls have been made in all types of weather with this apparatus and so far the results have been extremely satisfactory.

THE DEPTH-FLOWMETER

The depth-flowmeter is shown in Figs. 1 and 2. The instrument consists basically of a flowmeter, similar in many of its features to that described by Harvey (1934), in which has been incorporated a depth-recording unit which, like the depth gauges of Hermann (1949) and others, utilizes a Bourdon tube. Flow is measured by the number of revolutions of a smoked glass cylinder which is marked by a stylus, while depth is measured by the vertical displacement of the stylus arm.

Water flowing into the net turns a six-bladed rotor, and through a double worm gear train, giving a 100:1 reduction, the rotor drives a slotted metal barrel mounted vertically in a plain bearing. Both the vertical rotor shaft and the horizontal shaft are adjusted to run freely on hardened stainless steel points set in jewel cups. The blades of the rotor are set at an angle of 45° and the whole is finely balanced.

The smoked glass cylinders, on which the trace of depth and flow is marked by the phosphor-bronze point of the stylus arm, are a push fit on the metal barrel. The stylus arm is connected through a twin strip flexure system (Geary, 1954) made of beryllium-copper to the free end of the Bourdon tube. The fixed end of the tube, which is sealed with a brass plug, is securely mounted on the bottom bracket of the cast gun-metal meter frame. The Bourdon tube is sealed at normal atmospheric pressure so that increase of external pressure (with increased depth) causes the arc of the tube to decrease, resulting in a movement of the free end of the tube which produces a displacement of

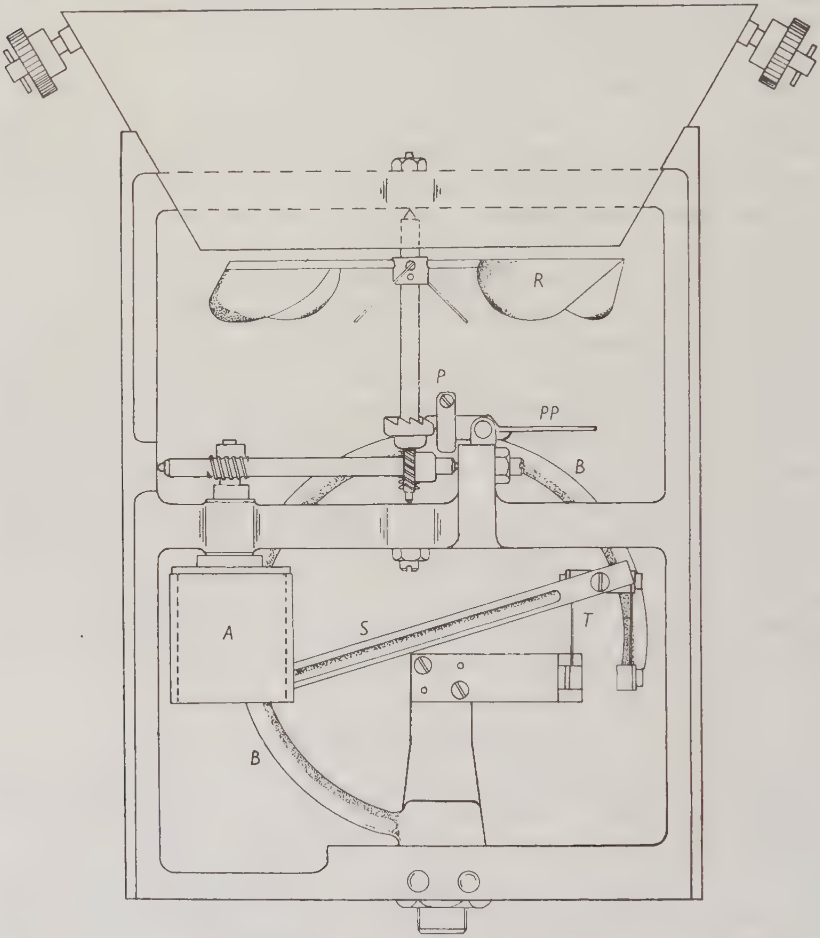

Fig. 1. The depth-flowmeter, one half actual size. *A*, smoked glass cylinder; *B*, Bourdon tube; *P*, pawl and ratchet mechanism; *PP*, pawl plate; *R*, rotor blade; *S*, stylus arm; *T*, twin strip flexure system.

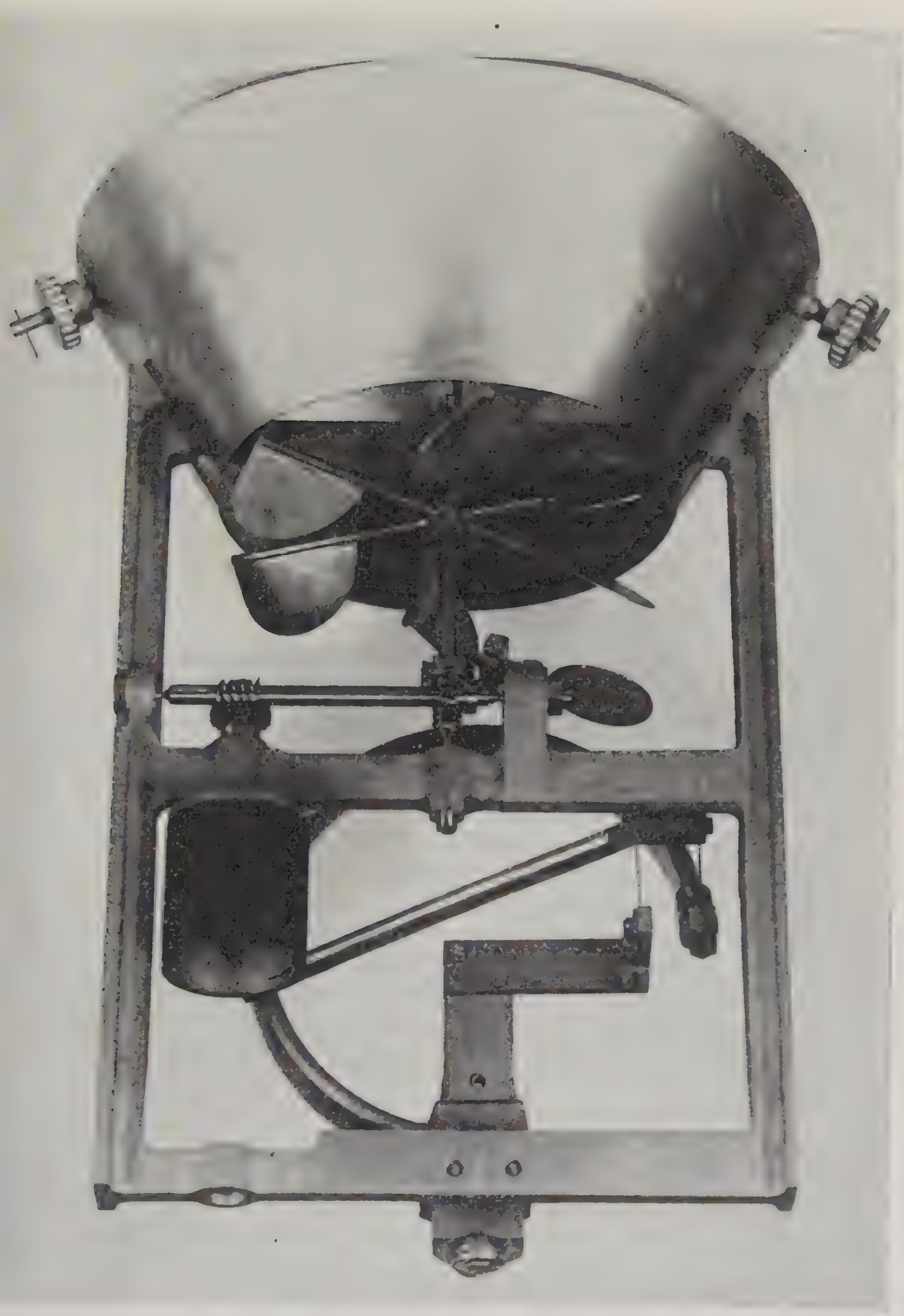

Fig. 2. The depth-flowmeter.

the stylus arm. In any haul, therefore, the maximum displacement of the stylus arm, which marks the smoked cylinder, is a measure of the maximum depth to which the meter has been lowered.

Bourdon tubes may be obtained to cover various depth ranges. We have used two meters, the deep one being fitted with a 2240 lb. Bourdon tube which gives a depth range of 0–1500 m, and the shallow one with a 400 lb. Bourdon tube which covers a depth range of 0–250 m. As the gear train is of the same ratio in each meter the shallow tube gives a greater vertical deflexion per revolution of the smoked cylinder and hence a very clear trace (see Fig. 5).

When the meter is hauled vertically through the water the rotor turns, causing the smoked glass cylinder to rotate past the point of the stylus arm thereby marking a trace on the smoked surface. At the same time as the meter is hauled through the water, the pressure (i.e. depth) is decreasing and so the stylus arm moves in an arc along the cylinder. The resultant of flow of water coupled with change in depth thus produces a helical trace in which the number of revolutions of the cylinder is proportional to the volume of water that has flowed through the meter, and the vertical displacement of the trace is a measure of the depth from which the meter has been hauled.

The instrument is designed to operate only as it is hauled through the water and a simple pawl and ratchet mechanism prevents the rotor turning as the meter is lowered either through the air or through the water. Flow of water (or air) against the bottom of the pawl plate forces the pawl, which is finely balanced by the position of a counter-weight, into engagement with a ratchet wheel on the rotor shaft, thereby preventing movement of the rotor and hence the smoked cylinder. On hauling, the direction of water flow on the pawl plate is reversed and the ratchet disengaged, allowing the rotor and the smoked cylinder to turn freely.

An inverted truncated cone[1] forms the mouth of the meter and also provides a means of attachment in the net. The meter is secured by the two knurled screws in a bayonet-type holder which is supported on three brass stays in the centre of a brass drum of 70 cm diameter. Fig. 3 shows the general arrangement of the net and the position of the drum and meter.

As protection against sea-water corrosion all the metal parts of the instrument, except for the pivots and the phosphor-bronze gears, are nickel plated. The weight of the meter in air is *ca* $1\frac{1}{3}$ kg and its total length is 24 cm.

THE NET

The net in which the flowmeter has been used (Fig. 3) is a modified version of the Nansen net. The anterior $\frac{1}{4}$ in. mesh netting collar has been replaced with a non-filtering cylinder of canvas. A suitable mounting behind the closing point was inserted to take the flowmeter, and the graded mesh of the filtering part of the Nansen net has been restricted to the one mesh throughout.

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[1] The truncated cone assists in directing an even velocity of water on to all the blades of the rotor.

The flowmeter net has a mouth diameter of 70 cm. Attached to the net ring is a cylindrical tube of canvas 4 ft. (122 cm) in length, around the lower end of which is sewn a piece of log line. This canvas tube slips over a brass drum, 70 cm diameter and 1 ft (30·5cm) in length, which carries the flowmeter, and the canvas is held on to the drum by a large metal band with a screw clamp which grips it immediately above the log line.

A similar fixture is provided for the attachment of the filtering part of the net at the lower end of the brass drum. The silk netting is sewn into a 6 in. (15 cm) wide band of canvas with a log line sewn around the top, and the first silk section is a 1 m cylindrical length of 74-mesh per linear inch bolting silk. At its after end it joins the second silk section which is a $1\frac{1}{2}$ m length of the same grade of silk tapering conically from a diameter of 70 cm to a diameter of 8 cm, where it joins a 3 in. (7·5 cm) wide canvas band that forms the attachment to the bucket. The bucket is of the type used with the 'Discovery' net, the N 70 V (Kemp, Hardy and Mackintosh, 1929).

The net is towed vertically by three bridles which are $\frac{1}{4}$ in. thick brass rods, 32 in. (81 cm) long joining together in a ring which fits into the release gear.

Supporting the flowmeter drum, there are three 3 mm phosphor bronze stays with brass eyes in the ends which are shackled to the net ring and to the flowmeter drum. These stays are seized to three of the six brass rings which are sewn around the anterior canvas collar, about 20 in. (50 cm) behind the net ring, to carry the throttling rope.

Three further stays of the same wire extend from the flowmeter drum down the whole length of the net. They are joined to the collar above the net bucket, and continue for about 3 ft. (92 cm) below the bucket to support the weight. A stout brass ring, 7 in. (17·8 cm) in diameter, encircles the net above the bucket, and is seized on to the three stays, to prevent their twisting.

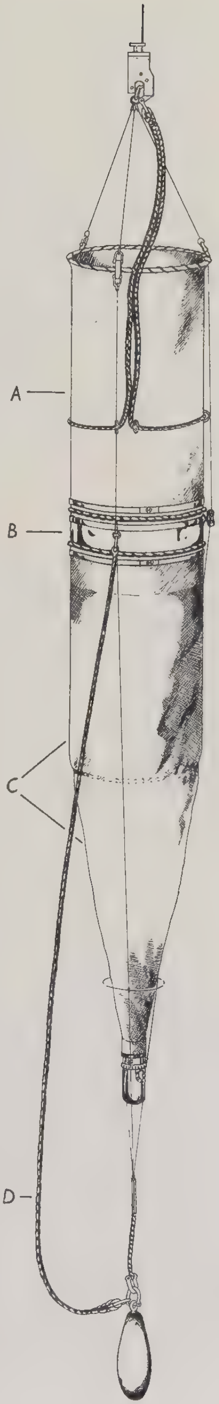

Fig. 3. The flowmeter net. *A*, canvas fore section encircled by closing rope; *B*, brass drum containing flowmeter; *C*, silk filtering section; *D*, handling line for weight.

The closing rope is a 16½ ft. (5·03 m) length of 2 in. (5 cm) rope. It passes round the net through the rings on the canvas collar and stay wires; its two ends are brought up and shackled to the closing mechanism.

As a fairly heavy weight (20–40 lb., 10–20 kg) is used, an additional line is attached to the weight to facilitate its handling at the surface. It is shackled to the side of the flowmeter drum. The three stays are attached to the weight by a snap link so that the weight can be detached easily and hooked up on the outboard side of the ship's rail.

The method of operation of this net is similar to that of the 'Discovery' net, the N70V (Kemp, Hardy & Mackintosh, 1929). Although the brass drum containing the flowmeter is somewhat bulky and heavy and may profitably be replaced by one made of fibre glass,* it not only affords protection to the meter, but makes it possible to pay out the net much faster than is possible with the standard N70V. This is a convenient saving of time on very deep hauls.

CALIBRATION OF THE METER

The meter must be calibrated both for depth and for flow of water before it can be used in the plankton net.

Depth. The flowmeter may be calibrated at sea by lowering it in the brass drum to various depths, having on the wire above it a reversing thermometer frame, or a reversing water-bottle, fitted with protected and unprotected thermometers. It is necessary to immobilize the smoked cylinder so that it does not rotate, by removing either the horizontal shaft or the rotor. The meter thus only records depth, giving a slightly curved trace, the vertical displacement of which is equal to the maximum depth to which the meter has been lowered. When the requisite amount of wire has been paid out, the thermometers are reversed. From the thermometer readings it is possible to calculate the actual depth of the meter and from the trace the vertical displacement of the stylus arm can be measured. The meter is lowered to different depths and the data used to construct a depth calibration curve. Fig. 4 shows such a curve for the deep meter which has been in use since 1954. As would be expected the relationship between depth and stylus displacement is linear, and from the slope of the curve it is seen that 1 mm stylus displacement is equivalent to a depth change of about 50 m.

A similar curve can be constructed for the shallow meter where 1 mm stylus displacement is equivalent to 7·5 m.

It should be mentioned that although it has been our practice to calibrate the meter at sea, it is also possible to do the calibration ashore in a pressure tank. The vertical displacement at different pressures gives a curve from which the depth per unit displacement can be calculated using the relationship that an increase in pressure of 1 atmosphere is equivalent to an increase in depth of about 10 m. Although this is a convenient method it is important to be able to check any calibration from time to time, particularly if any repairs or adjustments have been made to the instrument. Reversing thermometers provide a convenient and accurate method of doing this in the field.

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* The brass drum has since been replaced by one made of anodized aluminium alloy.

From experiments which have been performed in a pressure tank, it has been found that the accuracy of the depth reading from the flowmeter is limited, not by the accuracy of the Bourdon tubes, but rather by the accuracy with which the trace can be read. Under normal conditions with a clear trace we find this limit is ±0·25 mm, which corresponds to a depth of ±2 m with the shallow pattern flowmeter and ±15 m with the deep pattern flowmeter.

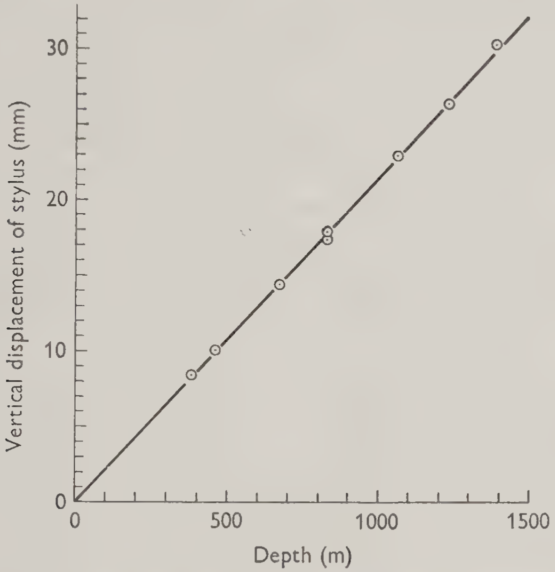

Fig. 4. Depth calibration of the 'deep' pattern flowmeter.

Flow. For similar reasons it is important to be able to calibrate the meter for flow of water at sea. The most practical way of doing this is to haul the meter, fitted in the brass drum with canvas forepart attached—but without the silk filtering section—vertically through a column of water of known length at a constant speed of 1 m/sec. It is then possible to relate the number of revolutions of the smoked cylinder to the flow past the meter of a volume of water equal to the length of the column times the area of the mouth of the net. This assumes that the axial velocity of the water (1 m/sec) is constant across the diameter of the canvas tube. This is not strictly true because the roughness of the walls of the tube (canvas and brass) exerts a drag on the water which results in a loss of velocity near the walls. For a canvas tube whose length is at least 40 times as great as its diameter it can be calculated (Goldstein, 1950) that the overall loss in the volume of water passing through the tube may be as great as 10%. In the case of the flowmeter net, however, the total length of the canvas tube and the brass drum is only about twice the diameter, and although no definite figure can be given it seems probable that the loss will be less than 2–3% and that it can safely be ignored. Furthermore, although the meter itself offers little resistance to the passage of water, it undoubtedly must modify the flow pattern to some extent. It is unlikely, however, that this will have a significant effect on the calibration. Both the net hauls and calibration are done with the meter in the same position and so it is only the absolute value of the calibration that the presence of the meter could affect. It is considered, however, that the meter lies sufficiently far behind the mouth of the net, for any obstructive effect to be resolved merely in an alteration of the velocity profile across the tube at the level at which the meter is placed, and that as far as the calibration is concerned its presence can safely be ignored.

The volume of a column of water of 50 m depth and cross-sectional area of 0·38 m^2 (which is the area of a 70 cm diameter mouth) is 19 m^3 and this is equivalent to the number of revolutions of the meter hauled through the same depth.

TABLE 1. FLOW CALIBRATION OF THE SHALLOW METER

Station	Depth (m.)	Revolutions	Revolutions per 50 m haul
3289	50–0	2·27	2·27
	100–0	4·67	2·33
	150–0	6·49	2·16
	200–0	8·16	2·04
3290	150–0	6·31	2·10
	200–0	8·06	2·02
3291	25–0	1·21	2·42
	75–0	3·41	2·28
	125–0	5·08	2·04
	175–0	6·86	1·96
	225–0	9·86	2·19
3292	50–0	2·16	2·16
	100–0	4·06	2·03

Mean revolutions per 50 m haul = 2·15

The results of thirteen calibration hauls made with the shallow meter are given in Table 1. The result of each haul has been expressed as the number of revolutions per standard haul of 50 m, and the calibration value is taken as the mean number of revolutions per 50 m haul. It is seen from Table I that the mean revolutions per 50 m haul is 2·15; which is equivalent to a flow of 19 m^3 of water.

THE PREPARATION OF THE SMOKED CYLINDERS AND TREATMENT OF THE TRACES

The glass cylinders on which the trace is recorded are made from precision-bored glass tubing of 28 mm bore, 2 mm wall thickness and 35 mm length. These cylinders cost about 1*s*. each but they can be used time and time again.

The problem of storing many smoked cylinders, as is the practice with bathythermograph slides, makes it more convenient to smoke the requisite number of cylinders just prior to use. The method is basically the same as that used for bathythermograph slides except that the cylinder is rotated in the smoky flame, and the deposit of carbon is made rather heavy. The procedure will be described in detail because it is essential to have a uniform deposit of soot which will not wash off as the meter is hauled through the water.

(1) If the cylinder has been used before, it is washed first in acetone to remove the cellulose varnish (see below) and secondly in carbon tetrachloride and then dried with a clean rag.

(2) A very thin even film of tallow (vaseline or petroleum jelly will also do) is applied to the outer surface of the cylinder.

(3) The cylinder is rotated at about 30 rev/min in the smoky part of a spirit flame, the spirit lamp being filled with a 15% solution of cellulose thinner in methylated spirit. A cylinder-holder geared to a small electric motor has proved particularly suitable for rotating the glass cylinder.

(4) Smoking is continued until the deposit is a fairly dense even black, which gives the best trace for photographic reproduction.

(5) After use the trace is preserved by first washing the cylinder in tap water, drying it, and then dipping it in clear cellulose varnish and drying again.

For ease of storage and also to facilitate the measurement of the flow and depth, a photographic record is then made of the trace. This is simply and quickly done by wrapping a piece of bromide photographic paper round the cylinder which is then illuminated from the inside. Even illumination is ensured by slipping the cylinder over a piece of Perspex rod which is slightly roughened on its surface. A torch bulb shines into the highly polished end of the rod. The resulting photograph, which is a reverse image of the trace, can be analysed and stored as a record of the haul, and the cylinder can be used again.

DESCRIPTION OF THE TRACES

The prototype flowmeter was first taken to sea in September 1954, and it gave most encouraging results. Only minor modifications were necessary, principally to improve the method of attachment of the meter inside the net, and since then it has been used successfully on four short cruises of the R.R.S. *Discovery II*. In all, about 160 hauls have been made, of which only three have yielded traces which were unreadable. The only major repair which has been necessary was the replacement of two jewelled cup bearings.

It has already been noted that the spiral traces obtained on the smoked-glass cylinders are photographed on to a piece of bromide paper wrapped round the outside of the cylinder. Fig. 5 shows a typical series of these photographic records of the traces. It will be clear from the manner in which they are made that the oblique lines in those figures represent one long continuous spiral. The traces which are shown for the 50–0, 100–50 and 200–100 m hauls were made with the shallow flowmeter, while those for the 500–200 and 1000–500 m were made with the deep pattern of flowmeter.

To understand the significance of the traces, let us first consider the one representing the 200–100 m haul at station 3312 (Fig. 5). The top of the trace represents the sea surface, and descending across the trace there is a gently

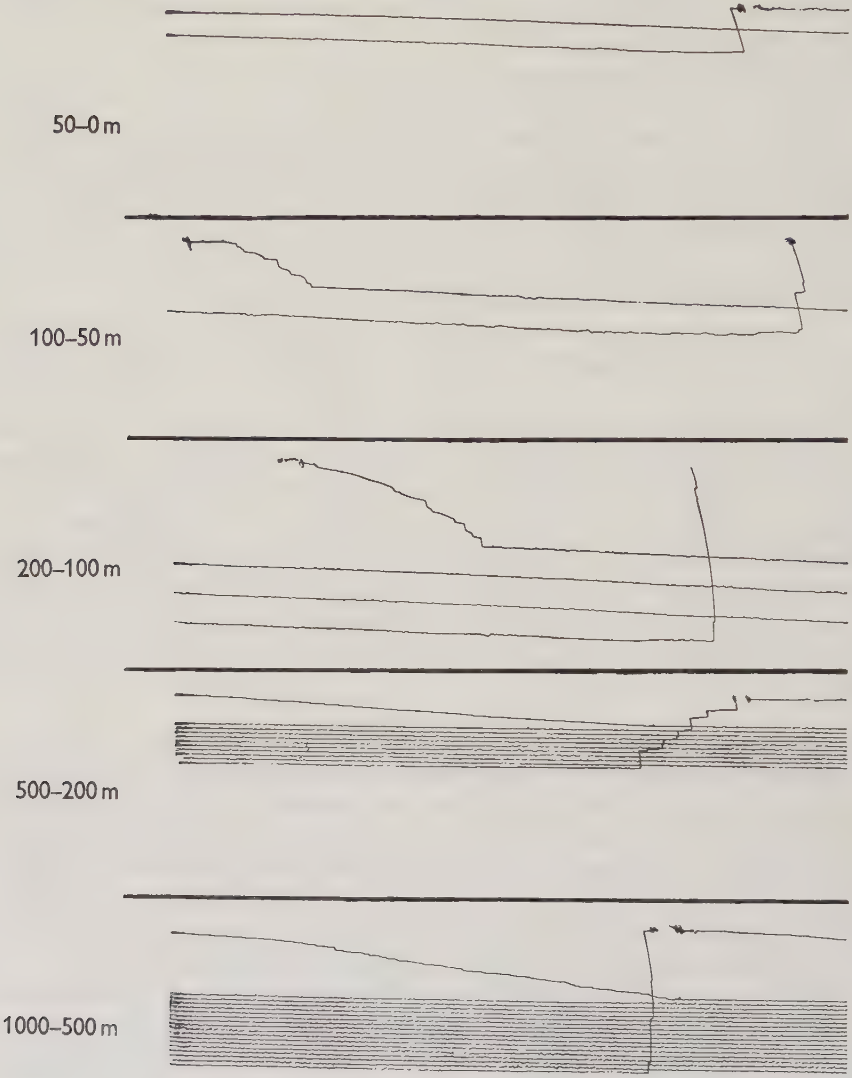

Fig. 5. Flowmeter traces, from hauls made at station 3312. ×0·86.

curved vertical line. This is the arc made by the stylus as the net was paid out. At this station the weather was very calm, and the net apparently went straight down without any surging which would be reflected in a rotation of the glass cylinder. From the lowest point of the trace, hauling was started, and the gently rising oblique line is formed as the glass cylinder is slowly turned by the rotor and the stylus rises with the decreasing pressure. In this particular trace, three complete rotations of the glass cylinder took place, and part way through the fourth revolution the net was closed and filtration stopped. The trace then rises upwards to begin with and then moves over to the left slightly as the closed net comes up to the surface. Theoretically the trace should have gone straight up to the surface after closure. This small amount of 'apparent filtration' which occurs after the net is closed is probably the result of a slight percolation of water through the canvas which is closing the net above the flowmeter. There must be quite a considerable pressure on this canvas as the closed net is hauled upwards, and no doubt a small amount of water is forced through the weave of the canvas.

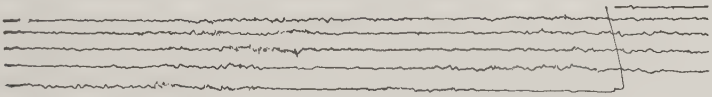

Fig. 6. Flowmeter trace from 100–0 m haul at station 3290, showing the effect of stray on the wire in the latter part of the haul.

From this explanation it will be easy to see what the other traces in Fig. 5 represent. The spiral trace made by the deep pattern flowmeter is of course much closer than that of the shallow version, since the vertical displacement of the stylus per unit of depth is not so great, but it is still quite clear to read. On most occasions it will be seen that there appears to be a certain amount of filtration when the net is lowered down. This is clearly seen, for example, in the 500–200 m trace in Fig. 5. Instead of the straight descending line as in the 200–100 m trace, there is a step-ladder effect on the 500–200 m trace. This is probably caused by water surging in and out of the mouth of the net since the rate of lowering cannot be adjusted to the movements of the ship. The authors observed this taking place under water and photographed it in the course of making a film of the closing of the Nansen net. It is unlikely that the net actually filters any appreciable quantity of water as it makes these surges, and if it did, anything it caught would be promptly washed out again.

A certain amount of short-period vibration can be seen in the traces, and this is probably caused by vibration of the stylus arm as the water flows over it.

So far, the hauls with the flowmeter net have been made on board the R.R.S. *Discovery II*, a ship which normally works stations hove to, into the wind. Consequently, the wire to the net is usually fairly near the vertical, all the time. At one or two stations, however, the ship has fallen off the wind in the middle of a haul with the result that the wire to the net has acquired a considerable angle of stray, and an examination of the traces from the flowmeter on these occasions has shown that the stray is reflected in the amount of water filtered by the net. In the 100–0 m haul at station 3290 a close inspection of the flowmeter trace (Fig. 6) shows that the spacing of the oblique lines becomes less nearer the surface. In other words, the amount of water filtered per unit depth near the surface was greater than the amount filtered per unit of depth, earlier in the haul. During this haul the wire took on a stray part-way through the haul. Obviously on such occasions the net is towed through the water as well as being hauled, and it filters more water per unit depth than it would in a truly vertical haul.

So far no traces have given any indication of reduction of filtration on account of clogging of the net by phytoplankton.

DISCUSSION

The hauls which have been made with the flowmeter net have come from a wide variety of localities: from the Faeroe–Shetland Channel, the English Channel, the Portuguese and Moroccan coastal waters, and in the open north-east Atlantic between 30 and 45° N. As mentioned above, no difficulty has been experienced with the net clogging, although many hauls have been made in waters visibly discoloured by the density of the phytoplankton, and also in areas of dense salp concentrations.

With the entire assembly of net and meter the measured flow of water, as indicated by the number of revolutions of the smoked cylinder, has been found to be only slightly less than the flow observed when the meter is hauled in the brass drum without the net attached, i.e. as in the calibration hauls (see p. 25). For instance the mean revolutions per 50 m haul for twelve shallow hauls with the complete net was found to be 1·96 compared with 2·15 given by the results in Table I. This difference in flow represents that part of the theoretical column of water that is prevented from flowing into the apparatus by the back pressure created by the presence of the silk netting, and can thus be used as a measure of the filtering efficiency of the net. Using the values quoted above we find that the filtration coefficient of the net when hauled at 1 m/sec has the surprisingly high value of about 90 %. This is, of course, on the fairly reasonable assumption that the presence of the net does not appreciably alter the pattern of flow that pertains in the drum during the calibration (i.e. when the net is absent). One point which no doubt contributes towards the high filtration coefficient is that the total length of the silk part of the net is $2\frac{1}{2}$ m, and as the first section is cylindrical the net has a very high ratio of filtering surface to mouth area.

As the filtration coefficient of the net is so high there will be little or no frontal wave preceding the net; this fact must add to the ability of the net to capture more active organisms: undoubtedly there will be less warning of approach of the net to them. The hauls which have been examined appear to take a very good representative sample of the bulk of the zooplankton. For a vertical net, the samples are relatively large, and this, of course, is clearly an advantage in attaining a reasonable degree of accuracy in their subsequent treatment.

The main advantage of the net, however, is its ability to sample different water layers in a quantitative manner. That this is essential in the study of the standing crop in the ocean is borne out by Foxton's (1956) results. These show that the extent of the seasonal change in the standing crop of zooplankton in the Antarctic could not have been properly understood without having divided hauls to a depth of at least 750 m available. Recent observations with the flowmeter net also bear out the fact that a large part of the standing crop is to be found in deep water. As an example, at station 3216 (April 1955) in the Faeroe–Shetland Channel, in a depth of 900 m, about 40% of the standing crop lay below 250 m and about 25% below 500 m. It would be possible to quote many more observations in subtropical waters where a similar distribution holds good.

The flowmeter itself may well find more extensive applications. Besides its adaptability to other vertical nets, it may be possible to use the meter on obliquely hauled nets. In this case it would probably only be possible to use the 'shallow pattern', as the spiral trace produced by an oblique tow of the 'deep pattern' meter would no doubt be too compressed to be interpreted accurately. The meter is also a useful tool for determining the filtration coefficients of other nets.

We should like to express our thanks to Mr R. Dobson, who was entirely responsible for producing the design of a working instrument from our inadequate sketches of the flowmeter. The meters were manufactured in the workshop of the National Institute of Oceanography. Mr R. Burt of R.R.S. *Discovery II* carried out all the rigging of the net and contributed many helpful practical ideas. Mr H. Charnock gave us guidance on matters concerning the water flow of the net. We are indebted to Mr P. M. David for the photograph of the depth-flowmeter in fig. 2, and also to Mr A. Style for the perspective drawing of the net in Fig. 3.

SUMMARY

The apparatus described in this paper was constructed for the purpose of sampling the zooplankton standing crop in the ocean in a quantitative manner.

A meter is described, which will record both the depth and amount of water which a net filters as it is hauled vertically through the water. The meter records on a smoked glass cylinder.

The meter is mounted in a brass drum which is positioned in front of the silk filtering section of the net. In front of the meter a long canvas sleeve carries the throttling rope which closes the net by the Nansen method.

Calibration of the meter for depth is done by using reversing thermometers and lowering to a series of suitable depths. Flow calibration is easily accomplished in the field by towing the canvas sleeve and meter without the silk net.

The method of smoking the glass cylinders and taking photographic records of them is described.

From the records it is possible to study the complete behaviour of the net below water—what depth range it has fished, how much clogging occurred, the depth of closure, etc.

REFERENCES

Clarke, G. L. & Bumpus, D. F., 1950. The plankton sampler—an instrument for quantitative plankton investigations. *Amer. Soc. Limnol. Oceanogr.*, Special Publication, no. 5. 8 pp.

Foxton, P., 1956. The distribution of the standing crop of zooplankton in the Southern ocean. *'Discovery' Rep.*, Vol. 28, pp. 191–236.

Geary, P. J., 1954. Flexure Devices. Pivots. Movements. Suspensions. *British Scientific Instrument Research Assoc., Res. Rep.*, M. 18.

Goldstein, S., 1950. *Modern Developments in Fluid Dynamics.* 2 vols. London: Oxford University Press.

Harvey, H. W., 1934. Measurement of phytoplankton population. *J. mar. biol. Ass. U.K.*, Vol. 19, pp. 761–74.

Hermann, F., 1949. A recording depth gauge for use in horizontal hauls with stramin nets and other pelagic fishing implements. *Medd. Komm. Havundersøg., Kbh., Ser. Fisk.*, Bd. 11, No. 5.

Kemp, S., Hardy, A. C. & Mackintosh, N. A., 1929. Discovery Investigations: Objects, equipment and methods. *'Discovery' Rep.*, Vol. 1, pp. 141–232.

Künne, Cl., 1929. Vergleich der Fangfähigkeit verschiedener Modelle von plankton-Netzen. *Rapp. Cons. Explor. Mer*, Vol. 59.

Nansen, F., 1915. Closing nets for vertical hauls and for horizontal towing. *Publ. Circ. Cons. Explor. Mer*, No. 67.

Wiborg, K. F., 1948. Experiments with the Clarke-Bumpus plankton sampler and with a plankton pump in the Lofoten area in northern Norway. *Fiskeridir. Skr. Havundersøk.*, Bd. 2, No. 2.

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NOTES ON *MYTILUS GALLOPROVINCIALIS* LAMARCK IN GREAT BRITAIN

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(Plate I and Text-figs. 1, 2)

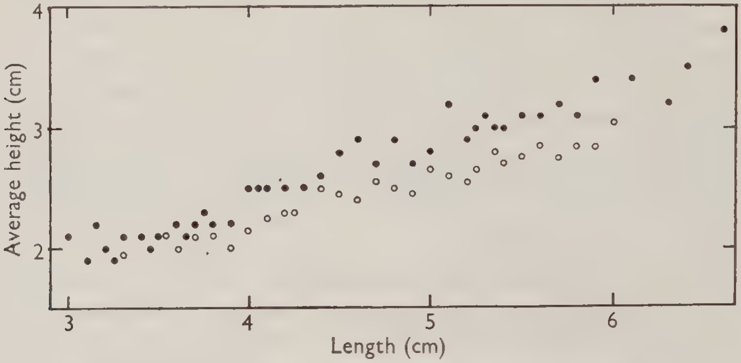
Mytilus galloprovincialis Lmk., the 'Mediterranean' mussel, was first reported, as *M. ungulatus* L., from Britain by Donovan (1802), who recorded that several specimens were found in Cornwall (for details of synonymy see below). He noted that it was known at that time as a Mediterranean but not hitherto as a British species. Jeffrey (1863) also recorded *M. ungulatus* L. from Cornwall and the Channel Islands, probably referring to *M. galloprovincialis* Lmk. This author also included a *M. galloprovincialis*, but this was not the mussel referred to in the present paper.

M. edulis var. *galloprovincialis* was recorded from several places in Cornwall in 1866 by a correspondent to the Journal of the Royal Institution of Cornwall. Sowerby (1887) recorded *M. edulis* var. *ungulata* L. from Cornwall and Guernsey and var. *galloprovincialis* from the English Channel. Tregellis (1896) recorded *M. edulis* var. *galloprovincialis* from Par, Falmouth, Helford and Hayle, and var. *ungulata* from Hayle and St Minver (all in Cornwall). Winckworth (1932) included the species in his list of British Marine Mollusca, but gave no locality. Dean recorded *M. galloprovincialis* as 'abundant in Cardiff Docks' (Conchological Soc., 1929), and Gardiner (1945) reported the species as present at Pendine, Carmarthenshire, and St Ives Bay and the Helford River, Cornwall.

In spite of these references to *M. galloprovincialis* in Britain, there seems to be no comprehensive account of the distribution of this species on our shores, or of its abundance relative to our common native mussel *M. edulis* L., and the present paper attempts to remedy these deficiencies.

The lack of information about this species was brought to light in the course of work on the mussel parasite *Mytilicola intestinalis* Steuer, when it was noticed that although the copepod often established a high level of infection in *Mytilus edulis* in north-west Europe, and frequently caused heavy mortalities, such infestations were less frequently reported from the Mediterranean and no disastrous mortalities are recorded. *Mytilicola* has been present in the Mediterranean for over 50 years (Steuer, 1902), but was not reported from north-west Europe until 1937 (Cole, 1951). These facts suggested that the Mediterranean mussel was in some way more resistant to the parasite than *Mytilus edulis*. If this were so, and if *M. galloprovincialis* would thrive in Britain, it might be of value for restocking mussel beds decimated by *Mytilicola*.

In 1952 Dr H. A. Cole drew my attention to a sample of mussels received from Padstow for bacteriological examination. These mussels differed in many respects from the normal *Mytilus edulis* of our shores. On examination it was found that 85 % of the mussel population at Padstow was of the 'unusual' type of mussel, the remaining 15 % being normal *M. edulis*. It was decided to investigate this 'Padstow-type' of mussel and to determine whether it was in fact *M. galloprovincialis* Lmk.



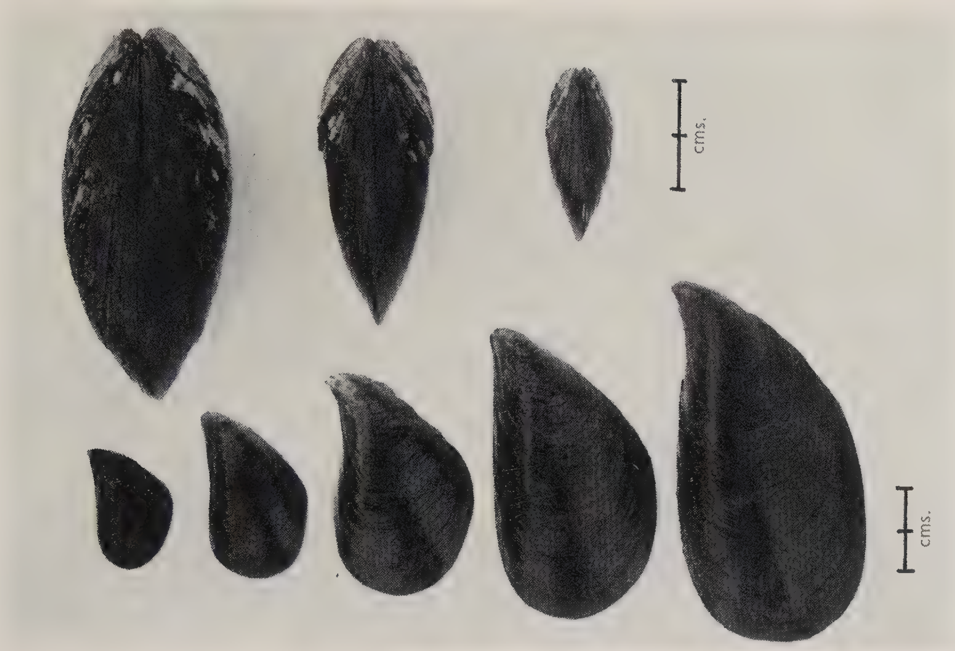
Text-fig. 1. Comparison of mean values of height with length for *Mytilus galloprovincialis* (●) and *M. edulis* (○).

THE 'PADSTOW-TYPE' MUSSEL

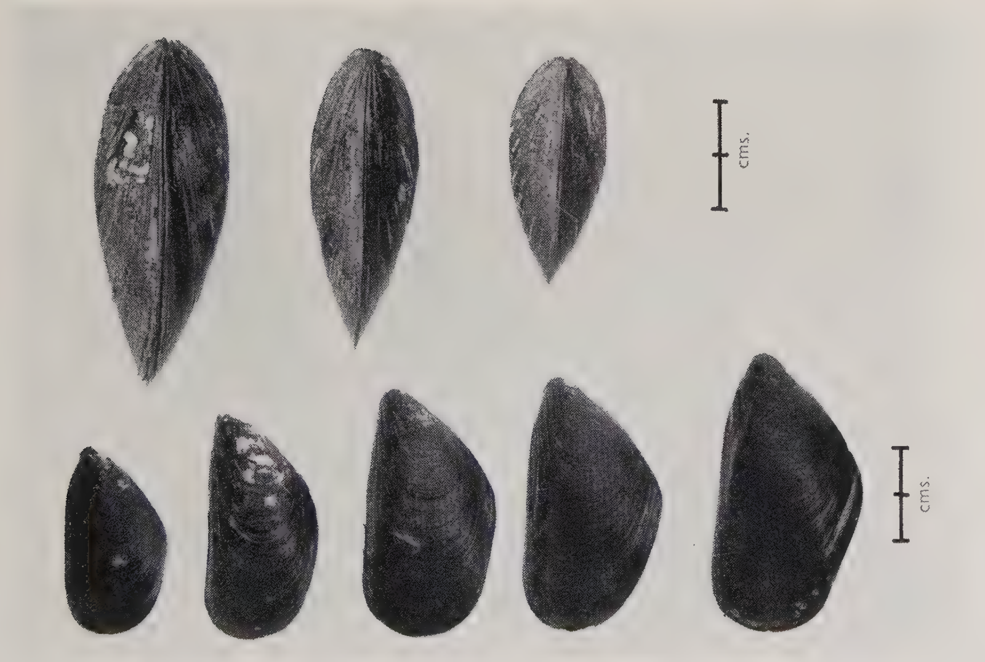
The mussels from Padstow were found to differ from *Mytilus edulis* in the following respects. (1) The umbones were more pronounced, more pointed and down-turned (see Pl. I). (2) The shells were proportionately higher, the length/height ratio for *M. edulis* being 1·95 and 1·77 for the Padstow mussels (see Text-fig. 1); these average figures were obtained from measurements of many specimens of each type. (3) The shells were less angular, and the distinct angle where the front and upper margins meet, which is often seen in *M. edulis*, was less evident in the Padstow mussels (see Pl. I). (4) They attained a larger size than *M. edulis*; individuals of 10–12 cm in length were quite common: in southern England *M. edulis* does not exceed 8–10 cm except under very favourable conditions. (5) The mantle edge was usually very dark, either blue, purple or violet, often appearing almost black, whereas in *M. edulis* it is much lighter, white to brown, usually appearing a straw colour.

EXPLANATION OF PLATE I

A. *Mytilus galloprovincialis* Lmk. from Padstow, Cornwall. Note the pronounced down-turned umbones, the high shells and the shape of the dorsal margin. B. *M. edulis* from Conway for comparison with A.



A



B

The literature on the Mytilidae appears to contain no detailed comparison of *M. edulis* and *M. galloprovincialis*, but the Padstow mussel seems to correspond to the description and figures of *M. galloprovincialis* given by List (1902) who describes the species in great detail. In both forms the umbones are pronounced and down-turned, the length/height ratio is very similar, being 1·77 for Padstow mussels and 1·8, calculated from List's measurements, for seventeen specimens of *M. galloprovincialis*, while the general shell outline of the Padstow mussel is similar to both the illustrations and descriptions given by List. The dark-coloured mantle edge of the Padstow mussel closely resembles that illustrated in colour by List for *M. galloprovincialis*.

Of other authors who have compared *M. edulis* and *M. galloprovincialis*, Sowerby (1887) states that *M. edulis* var. *galloprovincialis* is 'broader and flatter, beaks incurved', and gives an illustration of this mussel which is very similar in outline to the Padstow mussel, and appears larger than *M. edulis*. Forbes & Hanley (1848), referring to the mantle edge of mussels, state '...usually in British specimens a yellowish white colour though sometimes...tinged with brown and in the foreign variety *galloprovincialis* they are deeply tinged with purple'. Lambert (1950), in a brief comparison of *M. edulis* and *M. galloprovincialis*, states '...the latter is larger, wider and its umbone is more pronounced'. Width in this context is taken to mean shell height.

Since the Padstow mussel corresponds so closely to the descriptions of *M. galloprovincialis* and also resembles closely specimens of this species in the Norman collection of the British Museum and comes from an area in which Mediterranean species are known to occur (Yonge, 1949), it must be concluded that it is in fact *M. galloprovincialis* Lmk.

SYSTEMATICS

In the earlier literature there appears to have been some confusion between *M. ungulatus* L. and *M. galloprovincialis* Lmk. Lamy (1920) states that the two are synonymous, and List (1902) appears to have been of the same opinion. However, it appears that *M. ungulatus* is a form of *edulis*, and in fact Linnaeus (1758, 10th ed.) himself suggested this, and the figures to which he referred appear to be of distorted specimens of *M. edulis*. In the 12th edition of the *Systema Naturae* Linnaeus (1767) enlarged on his description of *M. ungulatus* given in the 10th edition. Lamarck (1819) appears to have been fully aware of both Linnaeus's *M. ungulatus* and *M. edulis* when describing *M. galloprovincialis*. Dodge (1952) considers *M. ungulatus* to be a species of doubtful validity and this appears to be the opinion of most conchologists at the present time. In view of this confusion it seems probable that the *M. ungulatus* which Jeffrey (1863) recorded from the coasts of Cornwall and the Channel Islands was in fact *M. galloprovincialis* Lmk. Donovan (1802) recorded *M. ungulatus* from the coast of Cornwall and his colour illustrations of this species so closely resemble in all respects the *M. galloprovincialis* taken from Cornwall, that it must be concluded that he was referring to the last-named species.

DISTRIBUTION

To study the distribution of *M. galloprovincialis* in Britain, and to determine whether stocks of this species were available for restocking mussel beds decimated by *Mytilicola*, surveys were made along the south-west peninsula of England, and on the south coast of Wales. *Mytilus galloprovincialis* was known to be absent from North Wales and the south coast of England from Teignmouth eastwards. At each site visited a sample of mussels was collected covering a wide range of sizes. The number of mussels varied according to quantities available. The mussels in each sample were sorted into groups of *M. edulis* and *M. galloprovincialis* on the criteria given above, and the percentage of the latter was calculated.

Results are shown in Table I, and in Text-fig. 2 the proportion of *M. galloprovincialis* and its distribution are shown.

It will be noticed that the mussel populations of north-west Devon and north and south-west Cornwall are dominated by *M. galloprovincialis*, with occasional specimens of this species occurring on the south coast as far east as Plymouth. In South Wales *M. galloprovincialis* occurs in small numbers at many points along the coast, being most common at Cardiff where it forms about half of the mussel population.

The absence of this mussel from the inlets of the Carmarthen coast can be explained on the grounds that it is intolerant of estuarine conditions (see below).

The well-defined zone of distribution of this mussel, with the rapid fall in the proportions of *M. galloprovincialis* over a short distance at the edges of the zone, suggests that this species would not be successful elsewhere in Britain. Although adult *M. galloprovincialis* have been kept in aquarium tanks at the laboratory at Burnham-on-Crouch in circulating River Crouch water for over a year, without any apparent ill effects, young specimens of 1–2 cm in length died within a few days of being placed in the River Crouch.

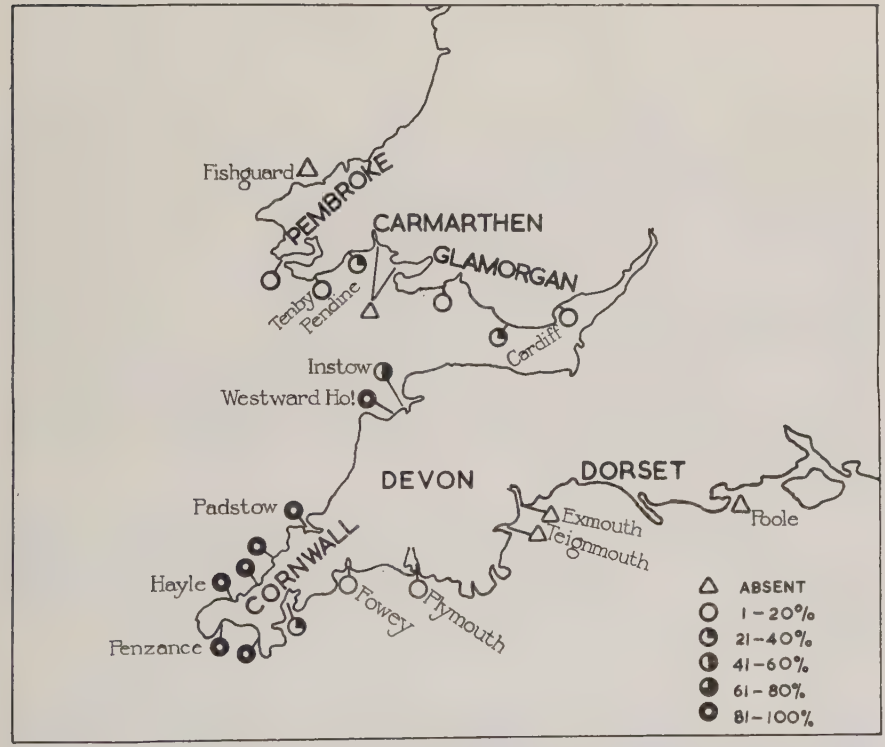
Some mussels received from Castletownbere in Southern Eire, collected by my colleague, Mr R. H. Baird, were found to be *M. galloprovincialis*, but it has not been possible to make a detailed study of its ecology in that area.

M. galloprovincialis has been reported from Concarneau, Finistère, by Bouxin (1955). Sinel (1906) mentions a *M. angulata*, 'the hoof mussel', and since he worked largely in the Channel Isles it may be assumed that the mussel he mentions was found there, and that he was in fact referring to *M. galloprovincialis*, '*angulata*' being a corruption of '*ungulatus*'.

TABLE 1. PERCENTAGE OF *MYTILUS GALLOPROVINCIALIS* AT SITES SURVEYED

Locality	No. in sample	*Mytilus galloprovincialis* (%)	Locality	No. in sample	*Mytilus galloprovincialis* (%)
Angle Bay	60	30	Newlyn	104	89
Tenby	147	1	Penzance	112	94
Pendine	56	40	Porthleven	68	87
Mumbles	72	13	Helford R.*	113	32
Aberthaw	45	26	Falmouth	260	75
Cardiff	110	60	Fowey	100	20
Instow*	20	35	Plymouth	200	2
Appledore	99	53			
Westward Ho!	58	88	Estuarine area of the Fal		
Padstow	180	85	Falmouth Harbour	260	75 Marine
Newquay	89	88	St Just	40	60
Gwithian	145	95	King Harry Reach	40	10 ↓
Hayle	73	97	Malpas	40	0 Brackish

* Estuarine areas


Fishguard
PEMBROKE
CARMARTHEN
GLAMORGAN
Tenby
Pendine
Cardiff
Instow
Westward Ho!
DEVON
DORSET
Padstow
CORNWALL
Hayle
Penzance
Fowey
Plymouth
Exmouth
Teignmouth
Poole
△ ABSENT
○ 1–20%
21–40%
41–60%
61–80%
81–100%

Text-fig. 2. Map of distribution of *Mytilus galloprovincialis* in Britain, showing the proportion of *M. galloprovincialis* in the mussel population.

ECOLOGY

M. galloprovincialis appears to occur in all types of habitat where salinity is not greatly reduced. On rocks on open exposed shores the mussel is stunted and thick-shelled, but at all times the characteristic features are maintained. In less turbulent waters, as in harbours and inlets, the mussel occurs in dense beds or clusters and grows to a very large size.

In surveys in the Fal and Truro rivers, and at Looe, *M. galloprovincialis* was found predominantly at the mouth of the estuary, being gradually replaced by *M. edulis* farther upstream (Table I). A similar situation was observed in the Camel estuary, at Padstow, but there the proportion of *M. edulis* was so small, and so very few mussels occurred in the less saline regions, that it would perhaps be unwise to draw any conclusions from this observation. It was noticeable that the few *M. galloprovincialis* found at Fowey and Plymouth were taken from the lower regions of the estuaries.

On a suitable substratum *M. galloprovincialis* appears to cover about the same vertical range as *M. edulis*.

RESISTANCE TO *MYTILICOLA*

In a field experiment at Poole, Dorset, in 1953, *Mytilus galloprovincialis* from Padstow, where *Mytilicola intestinalis* does not occur, were laid alongside *Mytilus edulis* from Conway, also free from *Mytilicola*, in a region where the local *Mytilus edulis* are heavily infected with the parasite. On examination 6 months later it was found that the Padstow mussels were infected to a level similar to or heavier than the Conway mussels, showing that the former possessed no immunity from infection. On the whole, however, the condition of the Padstow mussels seemed to be rather better than that of the Conway mussels with a similar level of infection (see Hepper, 1955). This is not in agreement with the results of Meyer Waarden & Mann (1954), who found that the condition of *M. galloprovincialis* was affected when two individuals of the parasite were present, whereas *M. edulis* began to lose condition only when infected with three individuals.

I am indebted to Mr G. L. Wilkins of the British Museum (Natural History) for helpful information concerning the systematics of *M. galloprovincialis* and for arranging for the examination of shells in the Norman Collection and a number of documents housed at the Museum.

SUMMARY

Mytilus galloprovincialis Lmk. is recorded as dominating the mussel population on the north coast of Devon and the north and south-west coasts of Cornwall from Instow to the Lizard. It was also found less frequently on the

coast of South Wales from Angle to Cardiff and on the south coast of Cornwall from the Lizard to Plymouth.

The chief distinguishing features of *M. galloprovincialis* from Cornwall are summarized, in comparison with *M. edulis* L.

M. galloprovincialis is apparently intolerant of estuarine conditions, but otherwise appears to be ecologically similar to *M. edulis.*

M. galloprovincialis is not resistant to infection by *Mytilicola intestinalis*, but appears to be less affected by the parasite than is *Mytilus edulis.*

The synonymy of the species is briefly discussed and it is concluded that, although earlier workers tended to confuse *M. ungulatus* L. (recorded also as *M. ungulata* and *M. angulata*) with *M. galloprovincialis* Lmk., the two types are distinct. *M. ungulatus* is a name of doubtful validity applied by Linnaeus to a distorted form of *M. edulis.*

REFERENCES

ANON., 1866. Cornish marine shells. *J. roy. Instn Cornwall*, Vol. 2, pp. 65–72.

BOUXIN, H., 1955. Observations sur le frai de *Mytilus edulis* var. *galloprovincialis* (Lmk.) *Rapp. Cons. Explor. Mer*, Vol. 140, pp. 41–4.

COLE, H. A., 1951. Le *Mytilicola* en Angleterre. *Rev. Trav. Off. Pêches marit.*, T. 17, Fasc. 2, pp. 59–61.

CONCHOLOGICAL SOCIETY, 1929. Recorder's Report (Marine molluscs). *J. Conch.*, Vol. 18, p. 300.

DODGE, H., 1952. A historical review of the mollusks of Linnaeus. Pt. I. The class Loricata and Pelecypoda. *Bull. Amer. Mus. nat. Hist.*, Vol. 100 (1).

DONOVAN, E., 1802. *The Natural History of British Shells.* London: F. and C. Rivington.

FORBES E. & HANLEY, S. 1848. *A History of British Mollusca.* London: John Van Voorst.

GARDINER, A. P., 1945. British marine records. *J. Conch.*, Vol. 22, p. 148.

HEPPER, B. T., 1955. Environmental factors governing the infection of mussels, *Mytilus edulis*, by *Mytilicola intestinalis. Fish. Invest. Lond.*, Ser. 2, Vol. 20, no. 3.

JEFFREY, J. G., 1863. *British Conchology.* London: John Van Voorst.

LAMARCK, J. B. M., 1819. *Histoire Naturelle des Animaux sans Vertèbres.* Paris.

LAMBERT, L., 1950. *Les Coquillages Comestibles.* Paris: Presses Universitaires de France.

LAMY, E., 1920. Notes sur les espèces de *Mytilus* décrites par Lamarck. *Bull. Mus. Hist. nat. Paris*, T. 26, pp. 520–6.

LINNAEUS, C., 1758. *Systema Naturae.* 10th ed. Stockholm.

—— 1767. *Systema Naturae.* 12th ed. Stockholm.

LIST, T., 1902. Die Mytilieden des Golfes von Neapel. *Fauna u. Flora Neapel*, Monogr. 27, 312 pp.

MEYER WAARDEN, P. F. & MANN, H., 1954. Untersuchungen über die Bestande von *Mytilus galloprovincialis* an der italienischen Küste auf ihren Befall mit *Mytilicola intestinalis* (Copepoda parasitica). *Boll. Pesca Piscic. Idrobiol.*, Ann. 29, N.S. Vol. 8, Fasc. 2, pp. 5–24.

SINEL, J., 1906. *An Outline of the Natural History of our Shores.* London: Swan Sonnenschein.

SOWERBY, G. B., 1887. *Illustrated Index of British Shells.* London: Sowerby and Simpkin Marshall.

STEUER, A., 1902. *Mytilicola intestinalis*, n.gen. n.sp., aus dem darme von *Mytilus galloprovincialis* Lam. *Zool. Anz.*, Bd. 25, p. 635.

TREGELLIS, J., 1896. The marine Mollusca of Cornwall. *J. Conch.*, Vol. 8, No. 6, pp. 191–200, 209–25.

WINCKWORTH, R., 1932. The British marine Mollusca. *J. Conch.*, Vol. 19, pp. 211–52.

YONGE, C. M., 1949. *The Sea Shore.* London: Collins, New Naturalist Series.

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THE GROWTH OF *SCROBICULARIA PLANA* (DA COSTA) IN THE GWENDRAETH ESTUARY

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(Text-figs. 1–3)

Scrobicularia plana (da Costa) is an abundant inhabitant of intertidal estuarine muds. Populations up to 1000 per m^2 have been recorded from the Tamar Estuary (Spooner & Moore, 1940). Similar population densities have been found in the Gwendraeth Estuary in South Wales. In one patch a population of 1025 per m^2 was found; the average length of the shells was 30 mm. Individual lengths up to 54 mm were found in other parts of the estuary. The large size of the specimens from the Gwendraeth prompted an investigation into the rate of growth and the age reached in this locality.

METHODS

Random samples of *Scrobicularia* were taken by digging up 1 m^2 to a depth of 30 cm in a region where the average population was 500 per m^2. A square metal frame with sides 0·5 m long and 15 cm deep was used to prevent mud slipping into the area being sampled. Four samples taken using such a frame made up the total sample of 1 m^2. Samples were collected in December 1953, April 1954, August 1954, April 1955 and July 1955.

The large specimens were sorted by hand and the small specimens by sieving. A sieve with a mesh of about 6 mm was used for the early samples, but for the last sample a 1 mm-mesh sieve was used. As there were some empty shells in the mud, care was taken to collect only the living animals; these were recognized in the field by the slightly protruding mantle lobes and by the presence of an oxidized layer of soil immediately next to the shell even when the animals were taken from a black layer. The specimens were washed and then boiled until the shells gaped widely and the animals dropped out. The shells were then washed and dried; one valve was kept from each. In this way a large number of shells could be stored and their lengths measured at leisure. The length of each shell, to the nearest mm, was written in pencil on its inner surface for future reference and size grouping. In all some 2500 shells were sorted and measured.

Like many other bivalved molluscs, *S. plana* has growth rings on its shell, but often only the last one or two are visible. The earlier growth rings become obliterated and only those left by the last few winters can be found. It is thus not possible to determine the age of a shell directly from its growth rings. It is, however, possible to deduce an approximate age by using a series of shells such as that shown in Fig. 1. By homologizing the rings on each shell with those on larger and smaller shells, and by working back to progressively smaller shells an estimation can be made of the age of any shell in the series. Examination of the growth rings on some of the large specimens was found to be facilitated by immersion in dilute hydrochloric acid for a short period. Immediately after such treatment the growth rings were very clear, but when the treated shells were stored the rings tended to disappear, and, of course, the treated shells were much more fragile than the untreated shells.

The environmental conditions in the sampling area were studied by measuring the salinity of the water over the area during a tide and by a grade analysis of the soil.

The salinity of the water was estimated with a glass hydrometer; the temperature of the water was measured at the same time and the density reading converted to salinity in ‰ using the graph given by Harvey (1945, fig. 12).

The sample of soil for analysis was taken by pushing a stout glass tube into the ground and then lifting it out after clearing the soil away from its sides. The ends of the tube were then closed with rubber bungs. The sample was taken about 3 h after the tide had uncovered the sampling area so that most of the superficial water had drained away. In the laboratory the soil was pushed out of the tube and chopped into 2 cm lengths which were weighed and then dried for 16 hr at 105° C. The subsequent treatment of the samples was identical with that of Holme (1949) except that the treatment with hydrochloric acid to remove carbonates was omitted and sieves with the following meshes were used: 30, 60, 90 and 100 I.M.M. In practice it was found that over 95 % of the soil passed through the 100 I.M.M. sieve.

CONDITIONS IN THE SAMPLING AREA

The area from which the samples were taken was about 70 m long, with the length parallel to the edge of the tide, and about 4 m wide. Neap tides covered the area for about 2 h; on a spring tide the time was increased to about 4 h. The depth of water over the surface of the mud on a neap tide was 0·5 m. The depth over the mud on a spring tide was not measured but is estimated at 2·0 m. The times given for coverage by the tide do not represent the limits of the time available to *Scrobicularia* for feeding since the surface is poorly drained and still wet for several hours after the tide has receded. The inhalant siphons of *Scrobicularia* can be seen actively sucking up the surface mud 3 or 4 h after being uncovered by the tide.

The salinity of the water over the area during a neap tide on 29 July 1955 is shown in Table 1. The salinity is probably somewhat higher than usual, due to the hot dry summer and the low state of the river. It was not practicable to measure the salinity during a spring tide, but a few measurements made during the winter 1952–53 showed a salinity of 18‰ at a level just above the sampling area about an hour before high tide. This somewhat lower salinity on a spring tide in winter when compared with a neap tide in summer indicates that the main factor governing salinity over the sampling area is the strength of flow of the river. Other measurements made during winter, as the tide was just uncovering the area, showed a salinity as low as 2‰.

TABLE 1. SALINITY OF THE WATER COVERING THE SAMPLING STATION DURING A NEAP TIDE IN JULY 1955

The tide covered the area at 12.44 G.M.T. and uncovered it at 14.45 G.M.T.

Time (G.M.T.)	Salinity (‰)	Time (G.M.T.)	Salinity (‰)
12.30	25	14.15	12
13.00	25	14.30	11
13.30	25	15.00	11
14.00	15		

TABLE 2. GRADE ANALYSIS OF THE SOIL IN THE SAMPLING AREA

Depth (cm.)	% water content	Fine sand as % dry weight	Silt + clay as % dry weight
1–2	31	75	24
2–4	28	78	21
4–6	30	80	19
6–8	29	81	17
8–10	27	84	13
10–12	26	88	10
12–14	24	90	8
14–16	24	90	8

Grade analysis of the soil showed that most of the particles passed through the 100 I.M.M. sieve and that of the total dry soil between 8 and 24% by weight belonged to the silt + clay fraction (Table 2). The most significant variation with depth is in the amount of silt + clay; the surface layers contain three times as much as the soil at a depth of 14–16 cm. The water content, as estimated by the present method, may not be quite the same as that of the mud *in situ*, but it does give a measure of the water-holding capacity of the soil. It is clear from Table 2 that this capacity increases with increasing silt + clay content, so that the surface layers have a higher water content.

AGE ESTIMATED FROM GROWTH RINGS

From a series such as that shown in Fig. 1 it is possible to construct a growth curve. Such a curve, based on the specimens in Fig. 1, is given by the broken line in Fig. 2. The middle part of the curve is based on several specimens at each length (Table 3). Other series of shells give slightly different curves, and if the whole range of variation is considered it is possible to draw up a table giving the upper and lower limits of length for a given year group

(Table 5). This range of variation, based on the December 1953 sample, is shown in Fig. 2. Attempts to gain greater precision in defining the growth curve do not seem to be very profitable. The details of the curve can only apply to this locality, and further examination of the shells with several growth rings often reveals a narrow interval between two wider ones, indicating that some years are better for growth than others.

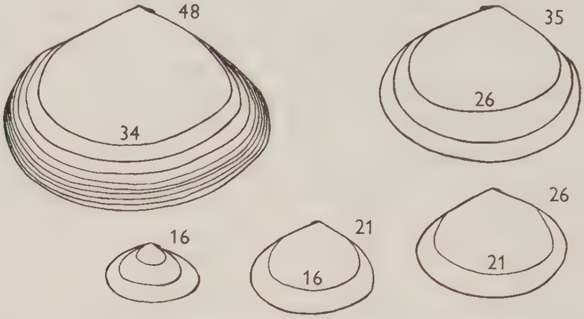

Fig. 1. *S. plana.* Series of shells used to construct the growth curve given by the broken line in Fig. 2. All are drawn to the same scale. The numbers above each shell and on some of the rings give the length in mm.

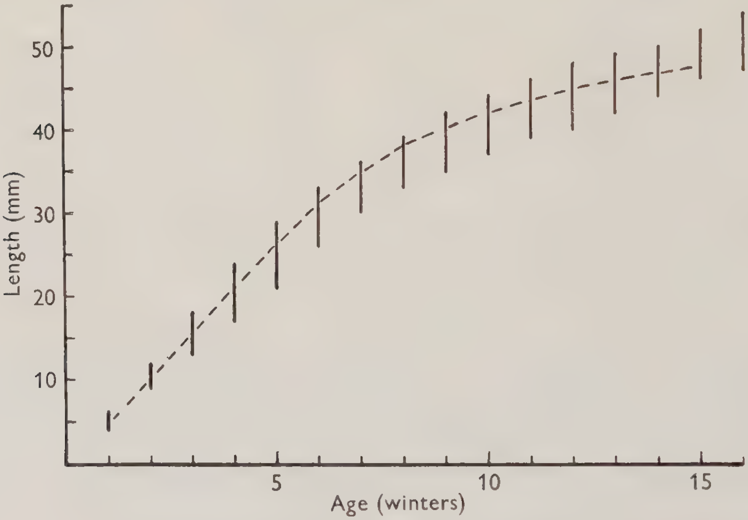

Fig. 2. *S. plana.* Growth curve constructed using the specimens in Fig. 1 (broken line), and the range of variation (shown by vertical lines) found in other series of shells.

If the mid-point in the range of variation given for each year group in Table 5 is taken as representative of an 'average' animal of that year the following summary can be given of the growth of *Scrobicularia*. A length of

5 mm is reached by the first winter. During each of the next two years another 5 mm is added to the length. Between the third and fourth winters 6 or 7 mm are added, thereafter the growth rate steadily decreases until the ninth or tenth winter, after which a fairly steady addition of about 2 mm per year is made.

TABLE 3. GROWTH RINGS ON MEDIUM-SIZED *SCROBICULARIA PLANA*

Sample collected in December 1953

Length of shell (mm)	No. examined	Mean length of growth rings (mm): Last	Mean length of growth rings (mm): Penultimate (when visible)
21	10	15·4	—
26	10	21·0	—
30	10	26·4	21·5 (4)*
35	10	32·2	26·5 (7)
38	5	35·0	30·0 (2)

* The numbers in parentheses give the number with two growth rings visible.

TABLE 4. GROWTH RINGS ON LARGE *SCROBICULARIA PLANA*

Sample collected in December 1953

Length 46 mm with 7 rings between one at 36 mm and the shell edge
Length 46 mm with 5 rings between one at 38 mm and the shell edge
Length 46 mm with 5 rings between one at 39 mm and the shell edge
Length 46 mm with 6 rings between one at 35 mm and the shell edge
Length 46 mm with 6 rings between one at 33 mm and the shell edge
Length 50 mm with 7 rings between one at 36 mm and the shell edge
Length 50 mm with 5 rings between one at 43 mm and the shell edge
Length 50 mm with 6 rings between one at 37 mm and the shell edge

TABLE 5. VARIATION IN LENGTH WITH AGE IN *SCROBICULARIA PLANA* FROM THE GWENDRAETH ESTUARY

Age (winters)	Length (mm)	Age (winters)	Length (mm)
1	4–6	9	35–42
2	9–12	10	37–44
3	13–18	11	39–46
4	17–24	12	40–48
5	21–29	13	42–49
6	26–33	14	44–50
7	30–36	15	46–52
8	33–39	16	47–54

Raymont (1955) has studied the early growth of *S. plana* in Kyle Scotnish, Scotland. Here the shells reach a length of 6 mm by the first autumn and 16 mm by the second. This is clearly a much greater increase during the second season than that found in the Gwendraeth, and may be due to the artificial fertilization of Kyle Scotnish.

Table 5 and Fig. 2 indicate that the age of shells above 20 mm in length cannot be determined accurately. For instance, a shell with a length of 33 mm might be 6, 7 or 8 years old. When even larger shells are considered the accuracy diminishes still further. A shell with a length of 46 mm may be anywhere between 11 and 15 years old. Some idea of the variation in large shells can be deduced from Table 4. Some individuals appear to live for about 18 years.

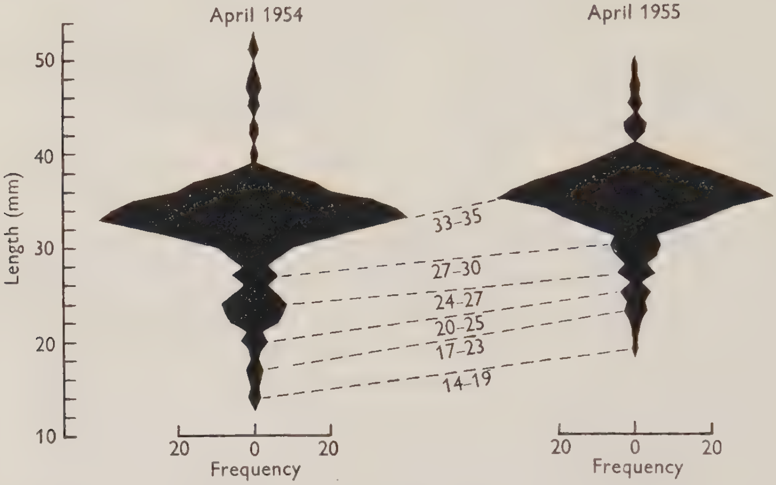

Fig. 3. *S. plana.* Shift in the peaks of length frequency distribution during one year.

LENGTH FREQUENCY DISTRIBUTION

In the preceding section it was assumed that the growth rings on the shell are annual rings. In order to test this assumption two samples were taken at a year's interval (April 1954 and 1955) to see how the peaks of the length frequency kites moved (Fig. 3). The shifts of the peaks are in fair agreement with the intervals between growth rings and all lie within the range of variation given in Table 5. If the mean size of the whole sample is considered there is a shift from 32·1 to 34·6 mm which is of a similar order to the increase in length between the seventh and eighth winters.

The earlier samples give no indication of the numbers of small shells so that no idea could be gained of the numbers of spat surviving. The July 1955 sample, taken with a 1 mm-mesh sieve, and sieved very gently so as not to destroy the delicate shells, showed a very small percentage of small shells. Only seven of the 483 shells in the sample were below 10 mm in length. This indicates that there is at present very little successful settlement of spat in the area. The dense adult population is probably the cause of this. Five hundred inhalant siphons working in a square metre would make the area a most difficult one for a recently metamorphosed *Scrobicularia* to survive in. Nevertheless, a few do survive, and it may be that when the present population bulge at a length of 30–40 mm dies out there will be heavier successful settlements of spat.

SUMMARY

Populations of *Scrobicularia plana* with densities up to 1025 per m^2 are found in the Gwendraeth Estuary.

In a region where the population density was about 500 per m^2 it was found that some specimens lived for 16–18 years and reached a length of 54 mm. An approximate growth curve is given.

There is very little successful settlement of spat in the sampling area; less than 2% of the shells were under 10 mm in length in July 1955. This low figure is attributed to the feeding activities of the dense adult population making the area difficult to settle in.

REFERENCES

Harvey, H. W., 1945. *Recent Advances in the Chemistry and Biology of Sea Water.* Cambridge University Press.

Holme, N. A., 1949. The fauna of sand and mud banks near the mouth of the Exe Estuary. *J. mar. biol. Ass. U.K.*, Vol. 29, pp. 189–237.

Raymont, J. E. G., 1955. The fauna of an intertidal mud flat. *Deep-Sea Res.*, suppl. to Vol. 3, pp. 178–203.

Spooner, G. M. & Moore, H. B., 1940. The ecology of the Tamar Estuary. VI. An account of the macrofauna of the intertidal muds. *J. mar. biol. Ass. U.K.*, Vol. 24, pp. 283–330.

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THE DISTRIBUTION OF POLYCHAETA IN OFFSHORE DEPOSITS IN THE IRISH SEA

By Eve C. Southward

Marine Biological Station, Port Erin[1]

(Text-figs. 1 and 2)

The bottom fauna of the Irish Sea around the Isle of Man and off the coast of Cumberland has been investigated by Dr N. S. Jones, who has described the fauna in general (1940, 1951, 1952) and the Amphipoda in particular (1948). Apart from the Amphipoda the smaller animals have remained relatively unknown. To extend this earlier work a survey of the polychaetous worms was carried out during 1952–54, and this paper deals with their distribution and ecology in the offshore bottom deposits. New records and details of taxonomic interest have been published elsewhere (Southward, 1956).

I am indebted to Dr N. S. Jones, Mr J. S. Colman and Dr A. J. Southward for advice and assistance; to Mr N. A. Holme for help with the mechanical analysis and to the University of Liverpool and the D.S.I.R. for financial assistance. The field work was carried out from the Marine Biological Station, Port Erin.

THE AREA INVESTIGATED

The area investigated during the 1952–54 survey was roughly the same as that studied by Jones (1951). To begin with all the collections were made within 10 miles of Port Erin but later the area was extended to include the deeper water to the west (Fig. 1).

The coastline of the southern half of the Isle of Man is steep and rocky and, except in the bays, the sea bottom slopes sharply from low-water mark to 10 fm. Below this depth the slope is more gradual and on the west side of the island the 50 fm. line is reached about 10 miles offshore. There is a channel about 70 fm. deep between the Isle of Man and Ireland. The sea to the south and east is shallower, the bottom is more irregular, and between the Isle of Man and England the depth rarely exceeds 20 fathoms.

The offshore grounds present a variety of habitats, most of which can be found within 10 miles of Port Erin. The deposits have been classified by Jones (1951) into four main types: (*a*) coarse sands, gravels including shell

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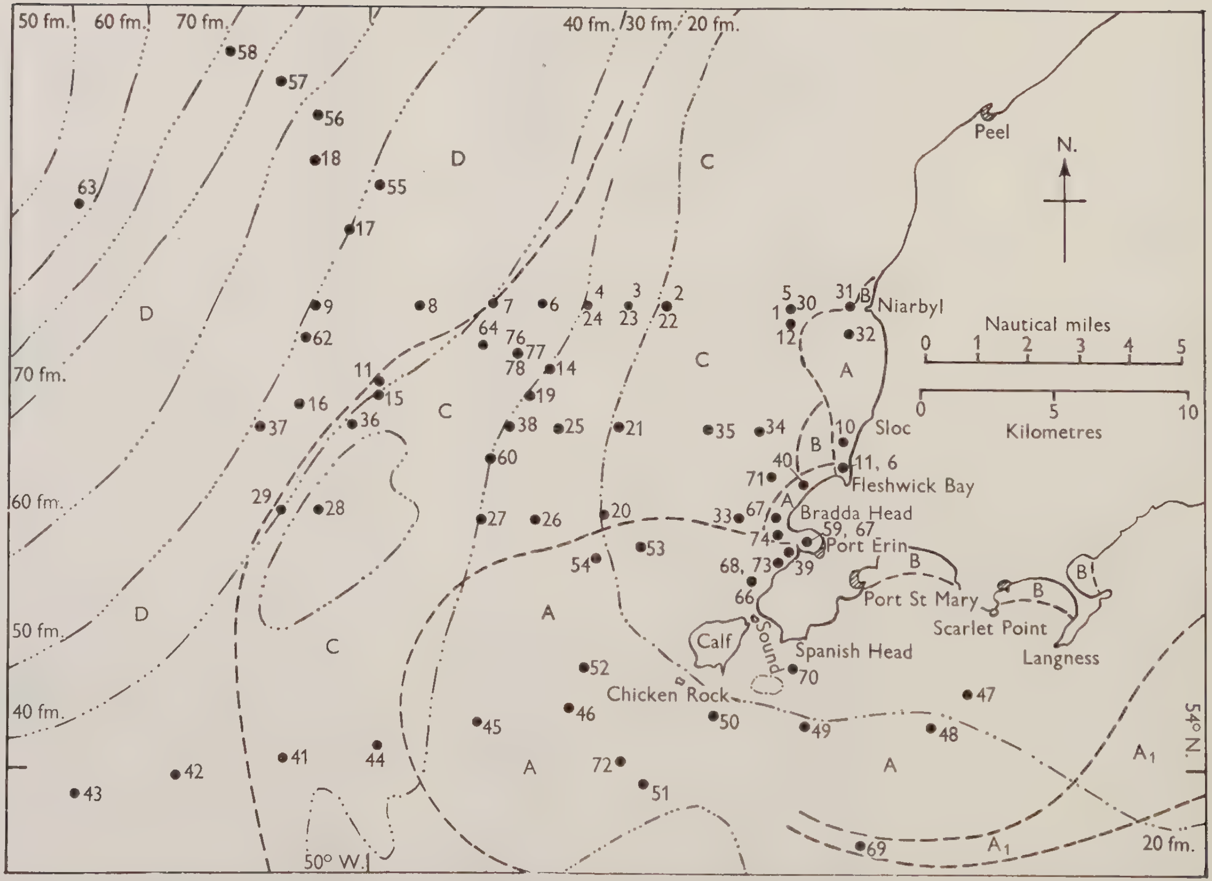
50 fm.
60 fm.
70 fm.
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Peel
N.
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Nautical miles
0 1 2 3 4 5
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Kilometres
Sloc
Fleshwick Bay
Bradda Head
Port Erin
Port St Mary
Scarlet Point
Langness
Spanish Head
Calf
Sound
Chicken Rock
54° N.
50° W.
A
A₁
B
C
D

Fig. 1. Map of the south end of the Isle of Man, showing positions of offshore stations (numbers as in Table II). The heavy broken lines mark the approximate limits of the various deposits. A gravels (A_1 *Modiolus* epifauna); B clean sand; C muddy sand; D mud.

and stony gravels; (*b*) fine sand; (*c*) muddy sand; (*d*) mud. The approximate limits of these types of deposit (as determined by Jones) are marked by the heavy broken lines in Fig. 1. The deposits grade into one another and there are no sharp boundaries between them.

In this area the main factor controlling the grade of a deposit appears to be the amount of water movement over it. The tidal currents reach 3½ knots at spring tides along the south coast of the island, and in places 4½ knots. There are weaker currents inshore along the west coast, but in a large area to the west of the island they are too slight to be perceptible (*West Coast of England Pilot*, 1948). It is in this area that soft deposits are present in relatively shallow water. Wave action may be felt down to 25 fm., as this is the least depth at which mud is found (Jones, 1951).

The surface temperature of the sea off the south-west coast had a mean annual range of 6·5° C during 1903–12, according to Matthews (1914). The monthly mean was lowest in February, at 7·5° C (all depths) and highest in August at 13° C. At this time of year the bottom temperature was less than 10° C; it reached its maximum in November when the temperature at all depths was 12° C. During 1953 (Slinn, 1956) the temperature was lower than the mean in February (6–7° C at all depths) and higher than the mean in August (13·5–15° C at the surface and 11·5° C at 50 fm.). The maximum bottom temperature was recorded in October (13·3–13·7° C).

The salinity of the sea in the same area is about 34‰ with an annual variation of about 0·2‰ (Matthews, 1914).

METHODS

GEAR USED

Several different types of gear were used for obtaining bottom samples. The most useful was a van Veen type of grab sampler taking a sample of one-tenth of a square metre (Thamdrup, 1938). The depth to which this instrument would dig depended on the hardness of the deposit. It was most efficient on mud and muddy sand, where it probably dug to at least 15 cm. Dredges were used on deposits too hard for the grab sampler. A small naturalist's dredge, with a bowed frame (1 ft. 6 in. wide) and a stramin bag, was used for fine gravel or gravel mixed with sand; judging from the fragments of *Ensis* sp. sometimes brought up it could dig 4–8 cm into these deposits. A larger naturalist's dredge (2 ft. 6 in.) was occasionally employed on sand and fine gravel but was less efficient than the smaller one. Where the bottom was composed of coarse gravel a scallop dredge (4 ft. wide and lined with ⅜ in. netting) was found most useful since the stramin of the small dredge was easily torn. It brought up only the coarse gravel and large stones, any fine material being washed out while the dredge was being hauled to the surface.

Depths were measured with an echo sounder and the positions of the stations calculated from compass bearings. A list of stations is given in Table 11, and their positions are marked on Fig. 1.

TREATMENT OF COLLECTIONS

The contents of the scallop dredge were examined on board the boat, larger specimens being picked out and samples of stones, shells and hydroids kept for more detailed examination. The samples taken in the naturalist's dredges were not sorted on board, but the whole or part of the sample was taken back to the laboratory. Grab samples were washed through a 2 mm round-holed sieve with a jet of water from a hose, on board the boat, after which the entire residue on the sieve was retained for examination in the laboratory.

At the laboratory samples and specimens were either examined fresh, soon after arrival, or preserved, without sorting, in 5% sea water formalin.

Weeds, hydroids and shells were searched carefully, usually under a dissecting microscope, and the worms picked out. Gravel was sorted in two stages: first, the larger animals were picked out by hand, then the gravel was washed several times and the washing waters strained through fine bolting silk. In this way many small animals were retained, that would otherwise have been lost. The first residue from the grab samples was treated in the same way if it contained much gravel. If not, it was sorted by hand.

In the case of the grab samples, and those of the small dredge, all the macrofauna was preserved and the animals other than polychaetes identified at least as far as phyla. When the scallop dredge was used only the polychaetes were picked out, but notes were made on the abundance of other animals.

After identification the polychaetes were preserved in 70% alcohol, and a representative collection of species has been stored at Port Erin.

GRADE ANALYSIS OF DEPOSITS

Small samples of most types of offshore deposit were taken from hauls of the grab sampler and small dredge. These samples were dried and later subjected to the method of grade analysis used by Holme (1954). This method separates the sample into eight grades of particles: (i) over 2 mm diameter; (ii) 2–1 mm; (iii) 1–0·5 mm; (iv) 0·5–0·25 mm; (v) 0·25–0·21 mm; (vi) 0·21–0·124 mm; (vii) 0·124–0·0313 mm; (viii) less than 0·0313 mm. In addition, fragments over 10 mm in diameter were separated from the first group.

TAXONOMY

The names and classification used here are mainly those used by Fauvel (1923, 1927). Some of the species identified during the present survey are not included by Fauvel; references to descriptions of these are given in the taxonomic paper referred to above (Southward, 1956).

BOTTOM DEPOSITS

During the field work bottom deposits were identified fairly readily as belonging to the four main groups described by Jones (1951), but several subgroups could also be recognized.

The coarse grounds (Fig. 1, A) include more than one type of gravel. A wide area to the south of the Isle of Man is occupied by large stones, shells and coarse shell gravel and the same type of coarse shell gravel also occurs on the west side of the island, off Bradda Head. Further north, between Fleshwick Bay and Niarbyl, there are deposits of fine stony gravel, which in places is mixed with larger stones or with nodules of coralline algae (*Lithothamnion* sp.). This algal deposit, mixed with muddy sand, also occurs to the west of Niarbyl.

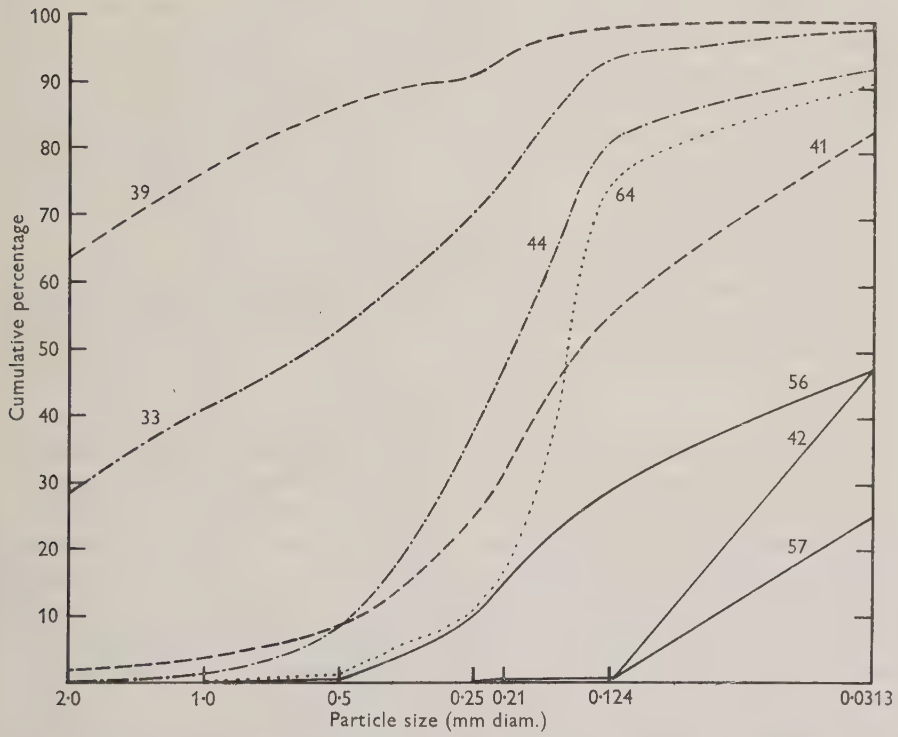

Fig. 2. The composition of various bottom deposits shown by means of cumulative curves. Station numbers as in Table 11. ----, fine stony gravel; —·—·—, muddy sand with shell gravel; ······, muddy sand; -----, muddy sand with *Turritella* shells; ———, mud.

Towards the outer limits of the coarse grounds the gravel is mixed with muddy sand, the proportion of which increases with depth until the pure muddy sand grounds are reached (Fig. 1, C). The muddy sand in turn grades into mud in deeper water, and along the edges of the mud grounds (Fig. 1, D) the muddy sand may contain large numbers of empty shells of *Turritella communis* Lamarck.

Mechanical analysis (see p. 52) of the deposits from fourteen stations has shown that the differences noticed in the field were definitely differences in grade composition. The composition of the various deposits (Table 1) can be shown graphically by means of cumulative curves (Fig. 2). The term 'muddy sand with shell gravel' refers to a wide range of deposits and the two curves (33 and 44) in Fig. 2 indicate the limits of gravel content among the samples analysed. The 'muddy sand with *Turritella* shells', referred to above, was found to contain more of the finer grades than 'muddy sand' proper, together with a small proportion of gravel derived from the *Turritella* shells. The 'muds' analysed contained 50% or more silt and clay (i.e. particles less than 0·0313 mm diameter).

THE FAUNA

The bottom fauna in general has already been described (Jones, 1951); only the polychaete fauna will be described here, and each type of deposit or habitat will be discussed separately.

THE MUDDY GROUNDS

The fauna of mud (Table 2) in the area investigated consisted mainly of burrowing and tubicolous animals. The polychaetes formed a high proportion (62%) of the population and the most common species were *Nephthys incisa*, *Glycera rouxi*, *Lumbriconereis hibernica* and *Tharyx marioni*. The first three of these are fairly active and probably predatory species (but see Sanders, 1956), while the cirratulid *Tharyx* is more sedentary in its habits, and apparently feeds directly on the deposit. Of the other fairly common species *Goniada maculata* is probably another predator, *Dasybranchus caducus* another deposit feeder, while *Spiophanes kröyeri* and *Prionospio malmgreni* inhabit more or less permanent mud tubes and probably feed by means of the ciliary currents of the tentacles. A few species seem to be confined to mud; these are *Leanira tetragona*, *Paraonis gracilis*, *Dasybranchus caducus*, *Clymene gracilis* and *Rhodine loveni*, but all except *Dasybranchus* were rare, even in mud.

Thus, the majority of the polychaetes found in mud were either active, predatory species or non-selective deposit feeders. Selective deposit feeders, such as the spionids, were few, and the specialized filter-feeding sabellids and serpulids were absent.

THE MUDDY SAND GROUNDS

Where the bottom deposit was muddy sand with little or no gravel the main members of the polychaete fauna were the same as those of mud, but the number of species present and the total population were much greater (Table 3). *Nephthys incisa* and *Glycera rouxi* were as abundant as in the mud, but *Lumbriconereis hibernica* and *Tharyx marioni* were rather less common. *Goniada maculata*, *Lumbriconereis gracilis*, *Spiophanes kröyeri*, *Chaetozone setosa*, *Diplocirrus glaucus*, *Owenia fusiformis*, *Myriochele heeri*, *Amphicteis gunneri*, *Trichobranchus glacialis* and *Terebellides stroemi* were all fairly common and characteristic species of this deposit. Most of these are sedentary species feeding on the deposit. *Owenia* has a crown of short ciliated tentacles with which it collects food as well as building its tube (Watson, 1900); *Myriochele* (a closely related species), though without tentacles, has a ciliated oral funnel with which it sets up a feeding current, and by closing this funnel it is able to reject unwanted particles. *Spiophanes*, *Diplocirrus*, *Amphicteis* and the terebellids probably feed by means of their ciliated tentacles (Blegvad, 1915; Hunt, 1925; Nicol, 1930; Mare, 1942; Dales, 1955). The cirratulid *Chaetozone* appears to feed directly on the deposit.

Among this largely burrowing and tubicolous polychaete population surface-living species were few, but probably all the aphroditids, except *Panthalis*, live on the surface of the deposit. Some of the tubicolous forms are dependent on sand grains or shell fragments for the formation of their tubes, for example *Owenia*, *Myriochele* and *Pectinaria* spp.; these are absent from mud and most common in the coarser muddy sands. Moreover, *Pectinaria* has a specialized method of feeding through a funnel in the deposit (Watson, 1928), which probably requires a fairly firm deposit for its construction.

MUDDY SAND WITH SHELL GRAVEL

Where shell gravel was mixed with the muddy sand the fauna was much richer, since it included most of the muddy sand species together with many of those from shell gravel; the number of species of Polychaeta (122) found in this deposit was greater than in any other investigated. The average density of the population was also fairly high (221 per m^2) though it formed less than 50% of the macrofauna. Most of the common species were burrowing or tubicolous, but with a greater gravel content the number of wandering and fixed species increased.

The most abundant species were *Lumbriconereis gracilis*, *L. hibernica* and *Owenia fusiformis*; the latter occurred at all stations and sometimes exceeded 100 per m^2 (Table 4). The other two were almost equally widely distributed but were less numerous. The other characteristic species of this mixed deposit were mainly burrowers and included *Nephthys incisa*, *Glycera rouxi*, *Goniada maculata*, *Eone nordmanni*, *Notocirrus scoticus* (all probably predators), *Laonice cirrata*, *Heterocirrus zetlandicus*, *Myriochele heeri*, *Amphicteis gunneri* and *Pectinaria auricoma* (deposit feeders of various types). The serpulid *Hydroides norvegica* was common on the larger shell fragments.

Polynoids, phyllodocids and syllids were more common in this mixed deposit than in muddy sand alone and many species of the two latter groups were found in crevices in shell fragments. The sabellid *Potamilla reniformis* was also found in crevices, two others (*Euchone rubrocincta* and *Chone suspecta*) were found, apparently free-living, among gravel.

THE FINE GRAVEL GROUNDS

Fine shell gravel

Of the fine gravels the fine shell had the richest fauna, and the polychaetes formed a higher proportion of the total macrofauna than in the other two. This deposit also contained the greatest number of polychaetes per square metre of any of the grounds investigated with the grab sampler, although this was the ground on which the grab obtained the smallest samples. Thus, the population may be considerably greater than that shown by this survey.

The most common polychaetes (Table 5) were *Pholoë minuta*, *Syllis armillaris*, *Glycera lapidum*, *Nematonereis unicornis*, *Laonice cirrata*, *Aonides paucibranchiata*, *Owenia fusiformis*, *Polycirrus denticulatus* and *Hydroides norvegica*. The first four of these are active and may be carnivorous, *Laonice* does not seem to form a permanent tube and its large eye-spots suggest that it also has an active mode of life, but its feeding is probably ciliary, as in other spionids. The only common tubicolous polychaete among the infauna was *Owenia fusiformis*. *Onuphis conchylega* and *Eunice harassi* were found occasionally among the epifauna; these two species build tubes of gravel and shell fragments but, unlike *Owenia*, they can move about, dragging their tubes with them (Watson, 1903).

Sabellids and serpulids were common in fine shell gravel, the serpulids being attached to shell fragments. These two groups appear to be most common where there is some water movement.

Fine stony gravel

The fine stony gravels provide a rather similar habitat to the shell gravel but the density of the population appears to be lower. However, as the grab sampler was not used, this conclusion is only tentative. The percentage of polychaetes in the total population was lower than in either of the other fine gravels. *Glycera lapidum*, *Capitomastus minimus*, *Aonides paucibranchiata* and *Pista cristata* were the most common species (Table 5), except at station 34, where the gravel was mixed with muddy sand and large stones. Here many of the species were more characteristic of muddy sand than of stony gravel. *Scalisetosus pellucidus* was probably commensal with the ophiuroid *Ophiothrix fragilis*, which was present in large numbers.

Coralline gravel

The 'coralline' gravels were composed of irregular nodules of a species of *Lithothamnion*, mixed with muddy sand. While some of the animals were characteristic of muddy sand, the fauna as a whole had more in common with that of the fine gravels. The polychaetes formed nearly 60% of the total macrofauna and the most common species were *Pholoë minuta*, *Lumbriconereis gracilis*, *Glycera lapidum*, *Nematonereis unicornis*, *Laonice cirrata*, *Capitomastus minimus* and *Pista cristata* (Table 5). *Lumbriconereis gracilis* appeared to be most common in mixed deposits of muddy sand and gravel, while *Capitomastus* and *Pista cristata* were more characteristic of stony gravels. The other species were common in fine shell gravel.

Thus, the polychaete fauna of all the fine gravels consisted mainly of active species, some burrowing (notably *Glycera lapidum*) and some living on the surface. Tubicolous species were few, except for the serpulids of the shell gravels. Few of the species feed directly on the deposit, without selection, and many of the active ones are probably predators.

THE COARSE GRAVEL GROUNDS

The fauna of coarse gravels appeared to be poorer than that of the fine gravels, but it is possible that further sampling and more careful examination of the gravel would reveal more species. Many tubicolous polychaetes were found in cracks and holes, but the external species were rather few and corresponded to the epifauna of the fine gravels. The common species of Polychaeta were mainly polynoids, sabellids and serpulids. *Halosydna gelatinosa* was fairly common, *Platynereis dumerilii* was frequent where there were fragments of algae among the gravel (it has been observed feeding on these), and the terebellid *Polymnia nebulosa* was common on large shell fragments. Both *Platynereis* and *Polymnia* inhabit semi-permanent mucous tubes. The serpulids occurred on shells and stones; *Pomatoceros triqueter* was the most common, being found on most shells and stones in every haul, while *Serpula vermicularis* and *Hydroides norvegica* were less common than *Pomatoceros* but nevertheless fairly frequent. *Sabellaria spinulosa* formed tubes of sand on shells and was widely distributed though not abundant.

Large stones had a surface fauna of *Pomatoceros* and *Hydroides* together with some polynoids, but the latter were more common in holes and crevices (Table 6). An examination of several lumps of limestone bored by *Hiatella* (station 50) showed the most common crevice-living species to be *Lepidonotus squamatus*, *Syllis armillaris*, *Autolytus aurantiacus* and *Polydora caeca*. The bigger holes in a large rock from station 74 contained many *Polymnia nebulosa* and several *Dasychone bombyx*, but the smaller species were not fully investigated. At station 32 the coarse stony gravel was mixed with muddy sand and the fauna as a whole was rather sparse, consisting mainly of muddy sand species, though the polynoid *Harmothoë impar* was common on the stones.

Crevices and holes in shells

The fauna of crevices in shells included many of the species found in holes in stones and also some boring forms. Of the polychaetes, only some species of *Polydora* and *Dodecaceria* are definitely known to bore into shells (e.g. Söderström, 1923), but it is possible some other species may do so. The burrows of *Polydora*, *Cliona* (the boring sponge) and *Phoronis ovalis* Wright were common in shells and shell fragments, and these were utilized by many other animals, some of which form their own tubes inside. The polychaetes appeared to be the most common animals in this habitat and thirty-two species were found (Table 7). The commonest and most widespread were *Syllis armillaris*, *Nematonereis unicornis*, *Polydora caeca*, *Polycirrus denticulatus* and *Potamilla reniformis*, but *Syllis variegata*, *Autolytus aurantiacus*, *Polydora ciliata*, *P. flava* and *Lumbriclymene minor* were sometimes common. Syllids and phyllodocids were abundant in this habitat but only one aphroditid, *Pholoë minuta*, was found. Oyster shells (*Ostrea edulis* L.) contained a large number of species but those of other molluscs (*Modiolus*, *Pecten* and *Glycimeris*) had a poorer fauna. Many of the species found in shells also inhabited the empty tubes of serpulids.

Hydroids

Several species of hydroids were common on shells and stones in the gravel deposits. They had a characteristic fauna, consisting mainly of polychaetes (Table 8), which differed from the gravel fauna. The serpulids *Hydroides norvegica* and *Spirorbis spirillum* were common, the latter often abundant. Many syllids lived among the branches, usually in their own mucous tubes, and several aphroditids were found, though some of these may have been inhabitants of the gravel on which the hydroids grew. Members of these two families may feed on the hydroids, but most of the other polychaetes are detritus—or filter—feeders.

Sponges

Sponges as well as hydroids are part of the epifauna of coarse gravel, and they also have a typical fauna. They were most frequent on and among the shells of *Modiolus modiolus* (L.), which are common in an area to the south-west of the Isle of Man (Fig. 1, A_1). This fauna has not been much investigated, but *Syllis spongicola* seems to be the most characteristic species. A few other syllids were found, notably *S. armillaris*, but they were also common species on hydroids and gravel.

THE CLEAN SAND GROUNDS

The offshore clean sand (Fig. 1, B) was sampled only in Port Erin Bay and off Niarbyl. A series of grab samples taken in Port Erin Bay (Table 9, station 59) indicated a fauna fairly rich in species of polychaetes and similar to that found at E.L.W.S. on the beach (c.f. Pirrie, Bruce & Moore, 1932; Moore, 1933). The grab sampler did not dig very deeply on this sand and the samples cannot have included the deeper burrowing animals; however, the number of animals per m^2 at 3–4 fm. exceeded the number found at E.L.W.S. on the beach. Two dredge hauls in the bay (65) brought up rather more surface-living species than the grab sampler. The common polychaetes, on or near the surface, were *Sthenelais limicola*, *Kefersteinia cirrata* and *Pista cristata*, while the common tubicolous and burrowing species were *Scoloplos armiger*, *Chaetozone setosa* and *Clymene oerstedii*. None of the three surface-living species has been found in the sandy beach, but *Scoloplos* is a characteristic intertidal species and *Clymene* is fairly common around and below M.L.W.S.

A small area of sand near Niarbyl was also sampled (31) and *Scoloplos armiger* and *Heterocirrus zetlandicus* were found to be common. Single specimens of *Nephthys cirrosa* were taken at Niarbyl and Port Erin, and the species may be common in the lower layers of the sand, which were not reached by either the grab sampler or the dredge.

DISCUSSION

The results of this survey indicate that many species of Polychaeta are widely distributed in the various types of habitat examined, but that, nevertheless, the polychaete fauna of each is distinctive. The density of the population varies very much with the type of deposit, as does the number of species present (Table 10). Environmental factors, apart from differences in the deposit, appear to have little effect on the fauna. Any direct effect of water movement on the fauna is masked by its effect on the composition of the deposit and these two factors cannot be separated. Differences in depth, unaccompanied by differences in the deposit, appear not to have much influence on the composition of the fauna. However, the depths in the area studied are comparatively small.

Most of the habitats examined were composite. That is, the softer deposits contained gravel and the gravels contained some sand or muddy sand. This may account for the wide distribution of some species. For example, seven species were found in all four main types of deposit but, of these, four glycerids, *Glycera rouxi*, *G. gigantea*, *Goniada maculata* and *Eone nordmanni*, are more characteristic of soft deposits and only occur in gravel where it is mixed with sand or muddy sand. *Nematonereis unicornis* occurs in all four deposits but is always associated with gravel, often living in crevices in shell fragments. The other two species, *Mystides limbata* and *Glycera convoluta*, are not common in any deposit and their distribution is rather sporadic.

The extent of the distribution of each species appears to depend mainly on its mode of life and feeding habits. Thus, carnivorous species are found in all habitats, but the burrowing forms, such as *Nephthys*, are restricted to the softer deposits, while the surface-living forms, such as the aphroditids, are more common on the harder grounds. Tubicolous species, such as *Owenia* and *Pectinaria*, are limited in their distribution by their dependence on definite sizes of particles for the formation of their tubes, while the serpulids require hard substrata for the attachment of their calcareous tubes. The distribution of filter-feeders may be limited by high concentrations of silt in the water, for no serpulids or sabellids were found in the mud, and few were found in the muddy sand (cf. Loosanoff & Tommers, 1948).

There were many examples of related species, of similar body form, being found in very different types of deposit. Thus, the Capitellidae were represented by *Dasybranchus caducus* in mud, *Notomastus latericeus* in muddy sand, *Capitomastus minimus* in fine gravel and *Capitella capitata* in sand. Again, the common species of *Lumbriconereis* (Eunicidae) in mud and muddy sand was *L. hibernica*, while *L. fragilis* was characteristic of coarse gravel and *L. gracilis* was common in the intermediate types of deposit. The factors involved in the distribution of these species may include the method of reproduction and the larval development and settlement (cf. Thorson, 1950; Wilson, 1952), as well as possible differences in feeding habits or food. Unfortunately, very little information is available on the reproduction of any species, since sampling was insufficient for detailed investigation of the breeding period or annual fluctuations in the population of the various species.

The numerical proportion of polychaetes was high in comparison with the other main faunistic groups at all stations where the animals were counted (Table 10). However, on the muddy grounds the heart-urchin *Brissopsis* and the crustacean *Calocaris* appear to form the greater part of the population by weight, though present in small numbers, and in the coarser grounds the Mollusca (or sometimes the Ophiuroidea) probably form the greatest proportion.

The density of the polychaete population on the grounds surveyed is low compared with that of some other British localities, and the species rarely attain the maximum size recorded by other authors. The maximum population found was 575 polychaetes per m^2 (station 19), and this compares badly with, for example, the 2000 per m^2 in the Rame mud near Plymouth (Mare, 1942).

The paucity of the fauna may be correlated with the low biomass found by Jones (1951, 1952) for the offshore grounds of the Isle of Man, compared with the grounds off the Cumberland coast. He considered that the low biomass was associated with a low run-off from the land in the area, and a low concentration of organic matter in the bottom deposits.

SUMMARY

During 1952–54 a survey was made of the Polychaeta living in the offshore bottom deposits of the south of the Isle of Man. These deposits range from stones and coarse gravel to soft mud; samples were obtained with a van Veen grab sampler and various dredges.

It was found that, although some species were widely distributed, each bottom deposit had a typical fauna, and that where the deposits graded into one another the polychaete fauna was also mixed. The distribution of each species appears to depend mainly on its mode of life and feeding habits; some can exist in several types of deposit or habitat, while others are very much restricted in their distribution.

The Polychaeta formed a high proportion, numerically, of the macrofauna, exceeding any other animal group, except possibly in the coarse gravels. However, the density of the polychaete population was low compared with other areas of the British Isles, and this may be correlated with the comparatively low biomass in the area.

REFERENCES

ADMIRALTY, HYDROGRAPHIC DEPARTMENT, 1948. *West Coast of England Pilot*. 9th ed. London.

BLEGVAD, H., 1915. Food and conditions of nourishment among the communities of invertebrate animals found on or in the sea bottom in Danish waters. *Rep. Danish biol. Sta.*, Vol. 22, pp. 43–78.

DALES, R. P., 1955. Feeding and digestion in terebellid polychaetes. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 55–79.

FAUVEL, P., 1923. Polychètes errantes. *Faune Fr.*, No. 5, 488 pp.

—— 1927. Polychètes sédentaires. *Faune Fr.*, No. 16, 494 pp.

HOLME, N. A., 1954. The ecology of British species of *Ensis*. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 145–72.

HUNT, O. D., 1925. The food of the bottom fauna of the Plymouth fishing grounds. *J. mar. biol. Ass. U.K.*, Vol. 13, pp. 560–99.

JONES, N. S., 1940. The distribution of the fauna and bottom deposits off Port Erin. *Proc. Lpool biol. Soc.*, Vol. 53, pp. 1–34.

—— 1948. The ecology of the Amphipoda of the south of the Isle of Man. *J. mar. biol. Ass. U.K.*, Vol. 27, pp. 400–39.

—— 1951. The bottom fauna off the south of the Isle of Man. *J. Anim. Ecol.*, Vol. 20, pp. 132–44.

—— 1952. The bottom fauna and the food of flatfish off the Cumberland coast. *J. Anim. Ecol.*, Vol. 21, pp. 182–205.

LOOSANOFF, V. L. & TOMMERS, F. D., 1948. Effect of suspended silt and other substances on rate of feeding of oysters. *Science*, Vol. 107, pp. 69–70.

MARE, M. F., 1942. A study of a marine benthic community, with special reference to the micro-organisms. *J. mar. biol. Ass. U.K.*, Vol. 25, pp. 517–554.

MATTHEWS, D. J., 1914. The salinity and temperature of the Irish Channel and the waters south of Ireland. *Sci. Invest. Fish. Br. Ire.*, Vol. 4, pp. 1–26.

MOORE, H. B., 1933. A comparison of the sand fauna of Port Erin Bay in 1900 and 1933. *Proc. malac. Soc. Lond.*, Vol. 20, pp. 285–294.

NICOL, E. A. T., 1930. The feeding mechanism, formation of the tube and physiology of digestion in *Sabella pavonina*. *Trans. roy. Soc. Edinb.*, Vol. 56, pp. 537–98.

PIRRIE, M. E., BRUCE, J. R. & MOORE, H. B., 1932. A quantitative study of the sandy beach at Port Erin. *J. mar. biol. Ass. U.K.*, Vol. 18, pp. 279–96.

SANDERS, H. L., 1956. The biology of marine bottom communities. Oceanography of Long Island Sound. X. *Bull. Bingham oceanogr. Coll.*, Vol. 15, pp. 345–414.

SLINN, D. J., 1956. Phosphate and oxygen in sea water off the Isle of Man during the years 1952–54. *Rep. mar. biol. Sta. Pt Erin*, No. 68, pp. 30–8.

SÖDERSTRÖM, A., 1923. Über das Bohren der *Polydora ciliata*. *Zool. Bidr. Uppsala*, Bd. 8, pp. 319–26.

SOUTHWARD, E. C., 1956. On some Polychaeta of the Isle of Man. *Ann. Mag. nat. Hist.* Ser. 12, Vol. 9, pp. 257–79.

THAMDRUP, H. M., 1938. Der van Veen-Bodengreifer. Vergleichsversuche über die Leistungsfähigkeit des van Veen- und des Petersen-Bodengreifers. *J. Cons. int. Explor. Mer*, Vol. 13, pp. 206–12.

THORSON, G., 1950. Reproductive and larval ecology of marine bottom invertebrates. *Biol. Rev.*, Vol. 25, pp. 1–45.

WATSON, A. T., 1900. On the structure and habits of the Polychaeta of the family Ammocharidae. *J. Linn. Soc. (Zool.)*, Vol. 48, pp. 230–60.

—— 1903. Observations on the habits of the Onuphidae and on the internal structures with which they fortify their homes. *Proc. Lpool biol. Soc.*, Vol. 17, pp. 303–18.

—— 1928. Observations on the habits and life history of *Pectinaria koreni* Malmgren. *Proc. Lpool biol. Soc.*, Vol. 42, pp. 25–60.

WILSON, D. P., 1952. The influence of the nature of the substratum on the metamorphosis of the larvae of marine animals, especially the larvae of *Ophelia bicornis*. *Ann. Inst. océangraph. Monaco*, T. 27, pp. 49–156.

TABLE 1. GRADE COMPOSITION OF SOME OFFSHORE DEPOSITS

	Particle size (mm diameter)										
Station	>10 %	10–2·0 %	2·0–1·0 %	1·0–0·5 %	0·5–0·25 %	0·25–0·21 %	0·21–0·124 %	0·214–0·0313 %	<0·0313 %	Type of deposit	Depth (fm.)
39	16·8	49·69	12·77	10·28	4·57	3·45	4·22	0·84	0·28	Fine stony gravel	5
33	14·17	14·592	12·633	11·972	17·934	5·432	17·618	4·407	1·253	Muddy sand and shell	17
35	6·2	2·737	1·935	5·895	28·228	11·790	30·609	8·225	4·380		18
60	0	0·592	3·069	8·375	30·538	12·549	33·850	5·385	5·639		31
45	0	6·070	9·844	9·811	21·766	9·926	23·571	9·734	9·269		32
44	0	0·020	1·270	6·803	29·468	10·143	32·542	12·623	7·127		34
64	0	0	0·165	1·437	9·125	6·139	58·652	15·126	9·349	Muddy sand	37
41	0	2·162	1·948	3·934	17·029	6·592	24·136	27·609	16·586	Muddy sand and *Turritella* shells	35
61	0	0·082	0·601	2·405	10·032	4·756	30·836	28·403	22·881		41
43	0	0	0·080	0·040	0·080	0·040	0·240	50·061	49·503	Mud	35
42	0	0	0·035	0·035	0·105	0·035	0·140	47·377	52·270		38
56	0	0·141	0·100	0·463	9·651	5·138	13·439	19·021	52·043		58
57	0	0·033	0·067	0·067	0·067	0·033	0·336	24·789	74·604		65
58	0	0·028	0·056	0·056	0·085	0·056	0·226	29·553	69·935		70

TABLE 2. POLYCHAET FAUNA OF MUD GROUNDS

(As number per square metre.)

Station number ...	*43*	*42*	*15*	*7*	*16*	*8*	*9*	*17*	*37*	*62*	*55*	*18*	*56*	*63*	*57*	*58*
Depth (fathoms) ...	35	38	40	40	45	45	50	50	50	50	53	55	58	60	65	70
Number of grab hauls ...	3	3	5	5	5	5	5	5	3	3	2	5	2	3	2	2
Species																
Panthalis oerstedi	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—
Leanira tetragona	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—
Mystides limbata	—	—	—	—	—	—	—	—	7	3	—	—	—	—	—	—
Ophiodromus flexuosus	—	—	—	—	—	—	2	—	—	—	—	—	5	—	—	—
Ancistrosyllis groenlandica	—	3	4	—	—	—	—	2	3	—	—	—	—	—	—	—
Nephthys incisa	7	20	18	10	10	12	2	12	23	10	15	2	15	7	—	15
Glycera convoluta	—	—	—	—	—	—	—	—	—	—	5	—	—	—	—	—
G. gigantea	—	—	—	—	—	—	2	2	—	—	—	—	—	—	—	—
G. rouxi	7	7	2	6	10	8	6	2	13	7	5	2	5	7	—	5
Goniada maculata	3	—	4	4	2	4	—	—	3	—	—	—	—	—	—	—
Eone nordmanni	—	—	4	—	—	—	—	—	—	—	—	—	—	—	—	—
Nematonereis unicornis	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—
Lumbriconereis hibernica	33	17	4	12	8	14	10	6	23	7	15	8	5	10	5	15
L. gracilis	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—
Spiophanes kroyeri	—	—	4	2	12	4	—	4	7	3	—	—	—	3	—	10
Polydora sp.	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—
Prionospio malmgreni	—	—	—	—	—	—	2	2	7	—	5	6	—	—	—	10
Paraonis gracilis	—	—	—	—	—	—	—	—	—	2	3	2	—	7	—	—
Tharyx marioni	13	10	—	4	12	18	22	6	20	13	15	6	25	37	20	35
Diplocirrus glaucus	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—
Scalibregma inflatum	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—
Dasybranchus caducus	—	3	6	4	2	10	—	2	3	3	—	—	—	—	10	—
Ammotrypane aulogaster	—	—	—	4	—	2	2	2	—	—	—	—	5	—	—	—
Clymene gracilis	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—
Rhodine loveni	—	—	—	—	—	—	—	2	—	—	—	—	5	7	10	—
Ampharete grubei	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—
Pectinaria sp.	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—

TABLE 3. POLYCHAET FAUNA OF MUDDY SAND

(As number per square metre.)

Station number ...	*3*	*23*	*4*	*24*	*19*	*6*	*41*	*64*	*29*	*36*	*61*
Depth (fathoms) ...	25	25	30	30	30	35	35	37	40	40	41
Number of grab samples ...	5	5	5	5	5	5	3	5	3	3	3
Species											
Aphrodite aculeata	2	—	2	—	—	—	—	—	—	—	—
Harmothoë antilopis	—	—	2	—	—	—	—	—	—	—	—
Lagisca extenuata	—	—	—	—	—	—	—	2	3	—	—
Polynoë kinbergi	—	—	2	—	—	—	—	—	—	—	—
Panthalis oerstedi	—	—	—	—	—	—	—	—	3	—	—
Phyllodoce mucosa	—	—	—	—	—	—	—	—	—	3	—
Eulalia sanguinea	—	—	—	—	2	—	—	2	—	—	—
Protomystides bidentata	2	—	—	—	—	—	—	—	—	—	—
Mystides limbata	—	—	—	—	—	—	3	—	3	7	3
Phyllodocidae: unidentified	—	—	—	—	—	—	—	2	—	—	—
Ophiodromus flexuosus	—	—	2	2	—	—	—	—	—	—	—
Hesionidae: unidentified	—	—	—	—	—	—	—	—	3	—	—
Ancistrosyllis groenlandica	—	2	—	6	2	4	—	2	—	—	—
Exogone gemmifera	—	—	—	—	2	—	—	—	—	—	—
Autolytus aurantiacus	—	2	—	—	—	—	—	—	—	—	—
Nephthys incisa	14	10	10	12	3	16	20	10	17	30	13
N. hombergi	—	2	2	—	—	—	—	—	—	—	—
Glycera rouxi	2	2	2	8	—	18	7	—	13	7	10

TABLE 3 (*continued*)

Station number ...	*3*	*23*	*4*	*24*	*19*	*6*	*41*	*64*	*29*	*36*	*61*
G. gigantea	—	—	—	—	—	2	—	—	—	—	—
G. convoluta	—	—	—	—	—	—	—	—	—	3	—
Goniada maculata	—	6	6	2	6	2	3	14	—	3	—
Eone nordmanni	—	—	—	—	—	4	—	—	—	7	—
Lumbriconereis hibernica	4	4	2	14	—	14	7	2	3	10	3
L. gracilis	6	8	2	6	4	2	7	2	—	—	—
Notocirrus scoticus	—	6	2	2	2	—	—	4	—	—	—
Drilonereis filum	—	—	—	—	—	2	—	—	—	—	—
Nerinides tridentata	—	2	—	2	—	2	—	2	—	3	—
Laonice cirrata	12	—	—	—	—	—	—	—	—	—	—
Spiophanes bombyx	—	10	—	—	4	6	—	—	—	3	—
S. kröyeri	—	14	12	10	24		10	2	—	3	3
Polydora flava	—	—	—	—	—	—	3	2	—	—	—
Prionospio malmgreni	—	10	—	6	2	6	—	6	—	10	—
P. steenstrupi	—	—	4	—	—	—	—	—	—	—	—
Aricidea branchiata	—	2	—	—	—	—	—	—	—	—	—
Paraonis lyra	—	—	—	—	—	—	3	—	—	—	—
P. gracilis	—	—	—	—	—	—	—	—	—	—	3
Chaetopterus variopedatus	—	—	—	—	—	—	—	2	—	—	—
Heterocirrus zetlandicus	—	—	—	—	4	—	—	8	—	—	—
Heterocirrus sp.	—	8	—	—	—	—	—	—	—	—	—
Chaetozone setosa	2	10	6	4	6	—	—	—	—	3	—
Tharyx marioni	—	2	—	6	2	4	10	2	10	20	43
Diplocirrus glaucus	2	4	4	—	10	—	3	8	—	13	—
Scalibregma inflatum	—	—	—	—	—	—	—	2	—	3	—
Notomastus latericeus	—	6	—	6	—	—	—	2	—	—	—
Dasybranchus caducus	—	—	—	—	—	—	—	—	—	—	3
Owenia fusiformis	26	192	16	2	386	—	—	12	—	—	—
Myriochele heeri	8	12	20	2	46	—	3	62	—	3	—
Ammotrypane aulogaster	2	—	—	—	—	—	—	—	—	—	—
Rhodine loveni	—	—	—	—	2	—	—	—	—	—	—
Clymene affinis	—	2	—	2	8	—	—	12	—	6	—
Clymene sp.	—	2	—	—	—	—	—	—	—	—	—
Leiochone clypeata	—	2	—	—	—	—	—	—	—	—	—
Ampharete grubei	—	—	—	2	6	—	—	—	—	3	—
Amphicteis gunneri	6	6	8	2	2	4	—	—	3	—	—
Sabellides octocirrata	—	—	—	—	4	—	—	—	—	—	—
Pectinaria auricoma	8	8	4	—	10	—	—	—	—	3	—
P. koreni	2	6	2	—	14	—	—	—	—	—	—
Pista cristata	—	2	—	—	—	—	—	—	—	—	—
Thelepus cincinnatus	—	—	—	—	2	—	—	—	—	—	—
Polycirrus denticulatus	—	—	2	—	—	2	—	—	—	—	—
P. plumosus	—	—	2	—	6	—	—	—	3	—	—
Trichobranches glacialis	—	10	10	—	6	2	—	2	—	7	—
Terebellides stroemi	2	10	8	2	2	—	—	4	—	7	—
Sabella pavonina	—	—	—	—	2	—	—	—	—	—	—

TABLE 4. POLYCHAET FAUNA OF MUDDY SAND MIXED WITH SHELL GRAVEL

(Number per square metre or relative abundance.)

Station number ...	*33*	*35*	*2*	*22*	*13*	*25*	*28*	*14*	*38*	*60*	*45*	*44*	*76*	*77*	*78*
Depth (fathoms) ...	17	18	20	20	20	25	29	30	30	31	32	34	35	35	35
Dredge (D) or grab (G) ...	D	D	G	G	G	G	G	G	G	G	D	D	G	G	G
Number of samples ...	1	1	5	4	5	5	3	5	3	3	1	1	10	10	10
Species															
Aphrodite aculeata	—	p	—	—	2	—	—	—	—	—	—	p	2	—	—
Lepidonotus squamatus	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—
Harmothoë impar	p	p	—	—	—	—	—	—	—	—	p	—	—	—	—

TABLE 4 (*continued*)

Station number ...	*33*	*35*	*2*	*22*	*13*	*25*	*28*	*14*	*38*	*60*	*45*	*44*	*76*	*77*	*78*
H. antilopis	—	—	6	—	—	—	7	2	—	—	—	—	1	—	2
H. haliaeti	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—
H. longisetis	—	p	—	3	—	2	—	—	—	—	—	—	—	—	1
H. sp.	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—
Lagisca extenuata	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Sthenelais minor	—	—	4	—	—	—	—	—	—	—	—	—	—	—	—
Pholoë minuta	p	p	2	3	2	—	3	—	—	—	p	—	—	—	—
Phyllodoce mucosa	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—
Eulalia bilineata	p	—	—	—	12	—	—	—	—	—	—	—	—	—	—
E. fucescens	p	p	—	—	—	—	—	—	—	—	—	—	—	—	—
E. sanguinea	p	—	2	—	—	2	—	—	—	—	—	—	—	—	2
E. macroceros	—	—	4	—	—	—	—	—	—	—	—	—	—	—	—
Notophyllum foliosum	—	—	—	3	—	—	—	—	—	—	p	—	—	—	—
Eteone longa	p	p	2	—	—	—	—	—	—	—	—	—	—	—	—
Protomystides bidentata	—	—	—	—	6	—	—	—	—	—	—	—	—	—	—
Mystides limbata	—	—	—	6	6	2	—	—	3	7	—	—	—	—	—
Phyllodocidae: unidentified	—	—	—	—	—	2	—	—	—	—	—	—	1	1	—
Podarke pallida	p	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Castalia punctata	p	p	—	5	—	2	—	—	—	—	—	—	—	—	—
Ancistrosyllis groenlandica	—	—	—	—	—	—	—	2	3	—	—	—	—	—	—
Syllis variegata	—	p	—	—	2	—	—	—	—	—	—	—	—	—	—
S. armillaris	c	—	4	—	28	—	—	—	—	—	p	—	—	—	—
S. cornuta	—	—	—	—	4	2	3	2	7	—	—	—	—	—	—
Eusyllis blomstrandi	—	p	—	3	—	—	—	—	—	—	—	—	—	—	—
Odontosyllis fulgurans	p	—	—	—	—	—	—	—	—	—	—	—	—	—	—
O. gibba	p	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Eurysyllis tuberculata	p	—	—	—	—	—	—	—	—	3	—	—	—	—	—
Exogone gemmifera	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—
E. verugera	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—
E. hebes	p	—	—	3	—	—	—	—	—	—	—	—	—	—	—
Sphaerosyllis hystrix	p	—	—	—	2	—	—	—	—	—	—	—	—	—	—
Autolytus aurantiacus	p	—	—	3	—	—	—	—	—	—	—	—	—	—	—
Nereis pelagica	—	p	—	—	—	—	—	—	—	—	—	—	—	—	—
Nephthys incisa	—	p	4	3	—	6	3	4	—	—	—	p	19	36	10
N. hombergi	—	—	—	—	—	—	—	—	—	—	—	p	—	—	—
N. rubella	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—
Ephesia gracilis	p	p	—	—	—	—	—	—	—	—	—	—	—	—	—
E. periphatus	p	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Glycera rouxi	—	p	—	5	12	—	27	2	13	—	p	p	6	2	3
G. gigantea	—	—	—	—	—	4	—	—	7	—	—	—	—	2	1
G. convoluta	—	p	6	—	—	—	—	—	—	—	—	p	—	—	—
G. lapidum	c	—	8	8	4	—	—	—	—	3	p	—	—	—	—
G. capitata	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—
Goniada maculata	—	p	—	3	6	—	27	—	7	—	p	c	6	2	9
G. norvegica	—	—	—	3	2	—	—	—	—	—	p	—	—	—	—
Eone nordmanni	p	p	4	3	2	—	—	2	—	—	—	p	2	1	3
Eunice harassi	p	p	—	—	—	—	3	—	—	—	—	p	—	—	1
Onuphis conchylega	p	p	—	3	—	2	3	2	7	—	—	p	—	—	3
Nematonereis unicornis	c	c	4	3	46	2	—	—	3	23	p	—	—	—	—
Lumbriconereis gracilis	c	c	4	20	12	4	13	10	33	7	—	p	9	1	6
L. latreilli	p	—	—	—	—	—	3	—	7	—	—	—	—	—	—
L. impatiens	—	—	—	—	—	—	—	4	—	—	—	—	—	—	—
L. hibernica	p	p	8	8	14	4	17	2	17	3	—	p	3	4	4
L. fragilis	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—
Notocirrus scoticus	—	p	2	8	4	—	23	6	7	—	—	p	5	—	4
Drilonereis filum	—	—	2	—	—	—	—	—	—	—	p	—	—	—	—
Eunicidae: unidentified	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—
Aricia cuvieri	—	—	—	—	—	—	—	—	3	—	p	—	—	1	—
Aricidea suecica	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—
A. minuta	—	—	—	—	—	—	—	—	—	—	—	p	—	—	—
A. branchiata	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—
Laonice cirrata	—	p	14	30	6	4	3	2	7	7	—	—	9	—	11

TABLE 4 (*continued*)

Station number ...	*33*	*35*	*2*	*22*	*13*	*25*	*28*	*14*	*38*	*60*	*45*	*44*	*76*	*77*	*78*
Aonides paucibranchiata	p	—	4	—	4	—	—	—	—	—	—	—	—	—	—
Spiophanes bombyx	—	—	—	—	4	2	—	8	—	—	—	—	4	—	—
S. kröyeri	—	—	—	8	—	6	23	—	3	—	p	—	4	1	5
Polydora flava	p	p	—	—	2	—	—	—	—	—	—	—	1	—	—
P. ciliata	—	—	—	—	14	—	—	—	—	—	—	—	—	—	—
P. caulleryi	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—
Polydora sp.	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—
Prionospio malmgreni	—	—	—	—	—	—	7	—	—	—	—	—	2	—	1
P. steenstrupi	—	—	—	—	—	—	—	2	—	—	—	—	2	—	2
Poecilochaetus serpens	—	—	—	3	—	—	—	—	—	—	p	—	1	1	—
Phyllochaetopterus socialis	—	p	—	—	—	—	—	—	—	—	—	—	—	—	—
Heterocirrus zetlandicus	c	c	—	8	32	4	3	—	3	7	p	p	1	—	—
H. caput-esocis	p	p	—	—	2	2	—	—	—	—	—	—	—	—	—
Tharyx marioni	—	—	—	8	2	2	13	—	3	1	p	—	—	1	—
Chaetozone setosa	—	—	6	3	2	2	3	6	—	—	—	—	10	—	11
Dodecaceria concharum	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—
Macrochaeta clavicornis	p	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Zeppelinia dentata	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—
Diplocirrus glaucus	—	—	—	—	—	2	—	2	3	—	—	—	1	7	7
Scalibregma inflatum	p	—	—	—	—	—	—	—	—	—	—	p	—	—	—
Notomastus latericeus	—	p	—	10	2	2	17	—	17	—	p	—	1	1	—
Owenia fusiformis	p	c	6	95	4	114	3	26	43	10	p	c	42	12	56
Myriochele heeri	—	p	—	13	2	24	40	8	3	—	—	p	4	6	42
Ammotrypane aulogaster	p	—	—	—	2	—	—	—	—	—	—	—	—	2	2
Clymene affinis	—	—	2	—	2	—	—	—	—	—	—	—	—	—	—
C. robusta	—	—	—	3	8	—	3	—	7	—	—	—	—	—	—
Clymene sp.	p	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nicomache lumbricalis	—	p	—	—	—	2	—	—	—	—	—	—	—	—	—
Praxillura longissima	—	p	—	—	—	—	—	—	—	—	—	—	—	—	—
Leiochone clypeata	—	—	—	—	4	—	—	—	—	—	—	—	—	—	—
Ampharete grubei	p	—	—	—	6	—	7	—	3	10	—	—	—	1	—
Amphicteis gunneri	—	p	2	20	10	2	10	8	7	—	p	—	9	—	15
Anobothrus gracilis	—	p	—	—	—	—	—	—	—	—	—	—	—	—	—
Sabellides octocirrata	—	—	—	—	2	—	—	4	—	—	—	—	—	—	—
Melinna cristata	—	p	—	—	—	—	—	2	—	—	p	—	—	—	—
M. palmata	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—
Sabellaria spinulosa	—	p	4	—	—	—	—	—	—	—	—	—	—	—	—
Pectinaria auricoma	—	p	4	—	2	6	—	34	10	—	—	c	23	10	16
P. koreni	—	—	—	5	—	2	—	8	3	—	—	p	7	—	—
Amphitrite gracilis	p	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Phisidia aurea	p	—	—	4	—	2	3	—	—	3	p	—	—	—	—
Pista cristata	—	p	—	20	—	2	—	—	7	—	p	—	—	—	—
Thelepus cincinnatus	—	—	—	3	6	—	—	—	—	—	—	—	—	2	2
Polycirrus denticulatus	p	—	—	5	—	—	—	—	—	—	—	—	1	—	—
P. plumosus	—	—	—	—	—	2	—	—	—	—	—	—	4	—	—
Amaea trilobata	—	—	—	—	—	—	—	—	—	—	p	—	—	—	—
Lysilla loveni	—	—	—	3	2	—	—	—	—	—	—	—	—	—	—
Trichobranchus glacialis	—	p	2	3	2	—	—	4	—	—	—	—	12	—	8
Terebellides stroemi	—	p	2	10	2	4	3	2	—	—	—	p	6	—	6
Terebellidae: unidentified	—	—	—	—	—	—	—	—	—	7	p	—	2	1	—
Sabella pavonina	—	—	—	—	20	—	—	12	—	—	p	—	—	—	—
Dasychone bombyx	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—
Potamilla reniformis	p	—	—	—	10	—	—	—	—	—	—	—	—	—	—
Chone suspecta	p	—	—	—	2	—	—	—	—	—	—	—	—	—	—
Euchone rubrocincta	p	p	—	10	2	4	—	—	3	—	—	—	—	—	—
Myxicola infundibulum	—	—	—	—	—	—	—	2	3	—	—	—	—	—	—
Serpula vermicularis	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—
Hydroides norvegica	p	—	—	15	22	2	3	2	13	20	p	p	—	—	1
Pomatoceros triqueter	p	—	4	—	—	—	—	—	—	—	p	—	—	—	—
Spirorbis spirillum	—	—	3	—	—	—	—	—	—	—	—	p	—	—	—

p = present c = common.

TABLE 5. POLYCHAET FAUNA OF FINE GRAVELS

(Expressed as numbers per square metre, or relative abundance.)

	Fine shell gravel					Fine stony gravel					Coralline gravel			
Station number ...	*71*	*20*	*21*	*26*	*27*	*75*	*10*	*11*	*39*	*34*	*1*	*5*	*12*	*30*
Depth (fathoms) ...	18	20	20	25	30	6	9	10	10	15	15	15	15	15
Dredge (D) or Grab (G)...	D	G	G	G	G	D	D	D	D	D	G	G	G	G
Number of hauls	1	3	3	5	3	1	1	1	1	1	4	5	5	1
Species														
Aphrodite aculeata	p	—	—	—	—	—	—	—	—	p	—	—	—	—
Malmgrenia castanea	—	—	—	2	—	—	—	—	—	—	—	—	2	—
Gattyana cirrosa	—	—	—	—	—	—	—	—	—	p	—	2	—	—
Lepidonotus squamatus	p	—	—	—	—	—	p	—	—	p	—	—	—	—
Harmothoë impar	p	17	7	—	—	—	—	—	—	—	—	—	—	—
H. antilopis	—	3	—	—	—	—	—	—	—	—	—	2	4	—
H. haliaeti	—	—	—	—	—	p	—	—	—	—	—	—	—	p
H. longisetis	—	—	—	—	—	p	p	—	—	—	—	—	—	—
H. lunulata	—	—	3	—	—	—	p	—	—	—	—	—	—	p
Harmothoë sp.	—	—	—	—	—	—	p	—	—	—	3	2	2	—
Halosydna gelatinosa	p	—	—	—	—	—	—	—	—	p	—	—	—	—
Lagisca extenuata	p	—	—	—	—	—	p	—	—	c	—	—	—	p
Scalisetosus pellucidus	—	—	—	—	—	—	—	—	—	c	—	—	—	—
Pholoë minuta	p	3	3	4	10	p	p	—	—	p	5	6	10	c
Pisione remota	—	—	—	—	—	—	p	p	—	—	—	—	2	—
Phyllodoce kosteriensis	—	—	—	—	—	—	—	—	—	p	—	—	—	—
Notophyllum foliosum	p	—	3	6	—	—	—	—	—	—	—	—	—	—
Eulalia viridis	—	—	—	4	—	—	—	—	—	—	—	—	—	p
E. bilineata	—	—	—	6	—	—	—	—	—	—	—	—	—	—
E. sanguinea	—	23	3	2	—	—	p	—	—	p	—	—	—	—
Eulalia sp.	—	—	—	—	3	—	—	p	—	—	—	—	—	—
Eteone foliosa	—	3	—	—	3	—	—	—	—	—	—	—	—	—
E. longa	—	—	—	—	—	—	—	—	p	—	—	—	—	—
Mystides limbata	—	—	7	—	—	—	p	—	—	—	—	—	—	—
Podarke pallida	—	—	—	—	—	—	—	—	—	p	—	—	—	p
Castalia punctata	—	7	63	10	—	—	—	—	—	p	—	—	—	p
Kefersteinia cirrata	—	—	—	—	—	—	p	—	—	p	—	—	—	p
Syllis armillaris	—	17	23	24	3	—	p	—	—	—	—	—	2	—
S. cornuta	—	7	—	4	—	—	—	—	—	—	—	2	2	—
Syllis sp.	—	—	—	6	—	—	—	—	—	—	—	—	—	—
Trypanosyllis coeliaca	—	3	—	6	—	p	—	—	—	—	—	—	—	—
Odontosyllis fulgurans	—	—	—	—	—	—	p	—	—	c	—	6	—	—
Eusyllis blomstrandi	p	13	—	—	—	—	—	—	—	—	—	—	—	p
Eurysyllis tuberculata	—	13	3	—	—	—	—	—	—	—	—	—	—	—
Exogone gemmifera	—	3	3	—	—	—	—	—	—	—	—	—	—	—
Sphaerosyllis hystrix	—	—	3	—	3	—	p	p	—	—	—	—	—	—
S. bulbosa	—	—	—	2	—	—	p	—	—	—	—	—	—	—
Autolytus rubropunctatus	—	—	3	—	—	—	—	—	—	—	—	—	—	—
A. prolifer	—	3	—	2	3	—	—	—	—	—	—	—	—	—
A. aurantiacus	—	—	—	—	—	—	—	—	—	—	—	8	—	—
Nereis zonata	—	7	—	—	—	—	—	—	—	—	—	—	—	—
N. pelagica	p	—	—	—	—	—	—	—	—	—	—	—	—	—
Platynereis dumerilii	p	—	—	—	—	—	—	—	—	—	—	—	—	—
Nephthys incisa	p	—	—	—	—	—	—	—	—	p	—	4	2	—
N. rubella	—	—	—	—	—	—	—	—	—	—	—	2	—	—
Ephesia gracilis	—	3	—	—	—	—	—	—	—	p	3	2	—	c
E. peripatus	—	—	3	—	—	p	p	—	—	—	—	—	2	p
Glycera lapidum	—	20	7	18	27	a	a	a	a	p	10	32	34	a
G. capitata	p	—	—	—	—	—	—	—	—	—	—	—	—	—
G. rouxi	—	—	—	—	3	p	—	—	—	p	3	2	6	p
G. gigantea	—	—	—	4	—	—	—	—	—	—	—	2	—	p
G. convoluta	—	—	—	—	—	—	—	—	—	c	—	—	—	—
Goniada maculata	—	—	—	—	3	—	—	—	—	—	—	2	—	p
Eone nordmanni	—	—	3	4	—	—	—	—	—	c	—	4	2	—

TABLE 5 (*continued*)

Station number ...	*71*	*20*	*21*	*26*	*27*	*75*	*10*	*11*	*39*	*34*	*1*	*5*	*12*	*30*
Eunice harassi	p	—	—	2	—	—	—	—	—	—	—	—	—	—
Onuphis conchylega	p	—	—	—	—	—	—	—	—	—	—	—	—	—
Nematonereis unicornis	—	10	20	14	10	—	p	—	—	p	35	18	10	p
Lumbriconereis gracilis	—	10	—	4	13	p	p	—	—	c	35	96	40	c
L. fragilis	—	—	—	2	—	p	p	—	—	—	—	2	2	c
L. hibernica	—	—	—	—	3	—	—	—	—	p	—	—	—	—
Drilonereis filum	—	—	—	—	—	—	—	—	—	—	—	—	2	—
Staurocephalus neglectus	—	—	—	—	—	p	p	p	—	—	—	—	—	—
Aricia cuvieri	—	3	—	—	—	—	—	—	—	—	—	—	4	—
Laonice cirrata	p	7	7	34	17	p	c	—	p	p	13	10	22	c
Aonides paucibranchiata	—	30	3	42	7	c	c	c	c	—	—	—	2	p
Spiophanes bombyx	—	—	—	—	—	—	—	—	p	p	—	—	—	—
Polydora ciliata	—	—	7	—	—	—	—	—	—	—	—	—	—	—
P. flava	—	3	—	2	—	—	—	—	—	—	—	—	—	—
P. caulleryi	—	—	—	2	—	—	p	—	—	—	—	—	—	—
Aricidea jeffreysi	—	—	—	4	—	—	—	—	—	—	3	—	—	—
A. branchiata	—	—	—	—	—	—	—	—	—	—	—	—	2	—
Paraonis lyra	—	—	—	2	—	—	—	—	—	—	—	—	6	—
Heterocirrus zetlandicus	—	—	26	4	—	—	—	—	—	c	—	4	—	—
H. caput-esocis	—	—	3	4	—	—	—	—	—	—	—	—	—	—
H. bioculatus	—	—	—	—	—	—	—	—	—	—	2	—	—	—
Tharyx marioni	—	—	3	2	3	—	—	—	—	p	—	—	—	—
Dodecaceria concharum	—	—	20	—	—	—	—	—	—	—	—	—	—	—
Macrochaeta clavicornis	—	3	—	—	—	—	p	—	—	—	—	—	10	p
Stylarioides plumosa	—	—	—	2	3	—	—	—	—	—	—	—	—	—
Diplocirrus glaucus	—	—	—	—	—	—	—	—	—	—	3	—	—	—
Flabelligera affinis	—	—	—	—	—	—	—	—	—	—	—	—	—	p
Scalibregma inflatum	—	—	—	—	—	—	—	—	—	p	—	2	2	p
Notomastus latericeus	—	—	—	—	—	p	p	p	—	—	—	2	—	p
Capitomastus minimus	—	3	—	—	—	c	a	c	—	a	40	48	50	—
Maldane sarsi	—	—	7	—	—	—	—	—	—	—	—	—	—	—
Clymene affinis	—	—	—	—	6	—	p	—	—	c	—	4	—	—
Leiochone clypeata	—	—	—	—	—	p	p	—	—	—	—	—	—	—
Nicomache trispinata	—	—	—	—	—	—	—	—	—	p	—	—	—	—
Maldanidae: unidentified	—	3	—	—	—	—	—	—	—	p	—	—	—	—
Owenia fusiformis	p	—	3	6	3	—	—	—	—	a	—	2	—	—
Myriochele heeri	—	—	—	—	—	—	—	—	—	p	—	—	—	—
Pectinaria auricoma	—	—	—	—	—	—	—	—	—	p	3	2	—	—
P. koreni	—	—	—	—	—	—	—	—	—	—	3	—	—	—
Sabellaria spinulosa	p	—	—	2	3	—	—	—	—	—	—	—	—	—
Ampharete grubei	p	—	—	—	3	—	—	—	—	p	—	—	—	—
Anobothrus gracilis	—	—	—	—	—	—	—	—	—	p	—	—	—	—
Amphicteis gunneri	p	—	—	—	—	—	—	—	—	—	—	—	2	p
Melinna palmata	—	—	—	—	—	—	—	—	—	—	8	—	—	—
M. cristata	—	—	—	—	—	—	—	—	—	—	—	—	—	p
Phisidia aurea	—	—	3	—	—	—	—	—	—	—	—	—	—	—
Pista cristata	—	—	—	2	—	a	a	c	a	p	3	10	22	c
Polymnia nebulosa	p	—	—	—	—	—	—	—	—	—	—	—	—	—
Nicolea zostericola	—	3	—	—	—	—	—	—	—	—	—	—	—	—
Thelepus cincinnatus	—	—	—	12	—	—	—	—	—	—	—	—	—	p
Polycirrus denticulatus	—	13	10	2	10	p	p	—	—	p	—	2	4	p
Trichobranchus glacialis	p	3	—	—	—	—	p	—	—	p	—	2	2	—
Terebellides stroemi	—	—	—	—	—	—	—	—	—	p	3	10	—	p
Lysilla loveni	—	3	—	—	—	—	—	—	—	—	—	—	—	—
Potamilla reniformis	—	—	27	8	3	—	—	—	—	p	—	—	—	—
Fabricia sabella	—	—	—	—	3	—	—	—	—	—	—	—	—	—
Jasminiera caudata	—	—	—	2	—	—	—	—	—	—	—	—	—	—
Chone suspecta	—	7	3	38	—	p	p	—	—	—	3	—	2	p
Euchone rubrocincta	—	—	—	—	3	—	—	—	—	—	—	—	2	—
Hydroides norvegica	—	17	57	26	3	—	—	—	—	p	—	—	—	p
Pomatoceros triqueter	—	30	3	38	—	—	p	—	—	a	—	—	—	—
Spirorbis spirillum	—	170	33	—	—	—	—	—	—	—	—	—	—	—

p = present c = common a = abundant.

TABLE 6. POLYCHAET FAUNA OF COARSE GRAVELS

	Shell gravel										Stones		
Station number ...	*67*	*40*	*47*	*48*	*53*	*54*	*52*	*46*	*69*	*72*	*74*	*32*	*50*
Depth (fathoms) ...	10	16	20	21	21	21	24	24	25	27	10	10	25
Species													
Hermione hystrix	p	—	—	—	—	—	—	—	—	—	—	—	—
Gattyana cirrosa	—	—	—	—	—	—	p	—	—	—	—	p	—
Lepidonotus squamatus	p	—	—	—	—	—	—	—	—	—	p	—	a
Harmothoë impar	—	—	—	—	—	—	—	—	—	p	—	a	—
H. haliaeti	—	—	—	—	—	—	—	—	—	—	—	p	—
H. reticulata	—	—	—	—	—	—	—	—	—	—	—	—	p
Halosydna gelatinosa	c	—	—	—	—	—	—	—	—	—	p	—	—
Lagisca extenuata	—	—	—	—	—	—	p	—	—	—	—	—	p
Polynoë scolopendrina	p	—	—	—	—	—	—	—	—	—	—	—	—
Scalisetosus assimilis	p	—	—	—	—	—	—	—	—	—	—	—	—
S. pellucidus	—	—	—	—	—	—	—	—	—	—	—	a	—
Pholoë minuta	—	—	—	—	—	—	—	—	—	—	—	—	p
Phyllodoce laminosa	—	—	—	—	—	—	—	—	—	p	—	—	—
Eulalia viridis	—	—	—	—	—	—	—	—	—	—	—	—	p
E. fucescens	—	—	—	—	—	—	—	—	—	—	—	p	p
E. macroceros	—	—	—	—	—	—	—	—	—	—	—	—	p
Castalia punctata	—	—	—	—	—	—	—	—	—	—	—	c	p
Kefersteinia cirrata	—	—	—	—	—	—	—	—	—	—	p	—	p
Syllis armillaris	p	—	—	—	—	—	—	—	—	p	—	—	a
S. variegata	—	—	—	—	—	—	—	—	—	—	—	—	p
Trypanosyllis coeliaca	—	—	—	—	—	—	—	—	—	—	—	p	—
Eusyllis blomstrandi	—	—	—	—	—	—	—	—	—	—	—	—	p
Exogone gemmifera	—	—	—	—	—	—	—	—	—	p	—	—	—
Autolytus pictus	—	—	—	—	—	—	—	—	—	—	—	—	c
A. rubropunctatus	—	—	—	—	—	—	—	—	—	—	—	—	p
A. longeferiens	—	—	—	—	—	—	—	—	—	—	—	—	p
A. aurantiacus	—	—	—	—	—	—	—	—	—	—	—	—	a
Autolytus sp.	—	—	—	—	—	—	—	—	—	p	—	—	p
Myrianida pinnigera	p	—	—	—	—	—	—	—	—	—	—	—	—
Nereis pelagica	—	—	—	—	—	—	—	—	—	—	—	—	p
Platynereis dumerilii	p	—	—	—	—	—	—	—	—	—	—	p	—
Nephthys incisa	—	—	—	—	—	—	—	—	—	—	—	p	—
Ephesia gracilis	—	—	—	—	—	—	—	—	—	—	—	p	—
Glycera lapidum	p	—	—	—	—	—	—	—	—	—	—	p	—
Goniada maculata	—	—	—	—	—	—	—	—	—	—	—	p	—
Eone nordmanni	—	—	—	—	—	—	—	—	—	—	—	p	—
Lumbriconereis fragilis	p	—	—	—	—	—	—	—	—	—	—	—	—
Polydora caeca	—	—	—	—	—	—	—	—	—	—	—	p	c
Chaetopterus variopedatus	—	—	—	—	—	—	p	p	—	—	—	—	—
Heterocirrus zetlandicus	—	—	—	—	—	—	—	—	—	—	—	p	—
Flabelligera affinis	—	—	—	—	—	—	—	—	—	c	—	p	—
Clymene robusta	—	—	—	—	—	—	—	—	—	—	—	p	—
Owenia fusiformis	—	—	—	—	—	—	—	—	—	—	—	c	—
Petta pusilla	—	—	—	—	p	—	—	—	—	—	—	—	—
Pectinaria auricoma	p	—	—	—	—	—	—	—	—	—	—	—	—
Sabellaria spinulosa	—	—	—	—	—	—	p	—	p	—	—	—	—
Polymnia nebulosa	p	—	—	—	—	—	p	c	—	—	c	—	—
Pista cristata	—	—	—	—	—	—	—	—	—	—	p	—	—
Polycirrus denticulatus	—	—	—	—	—	—	—	—	—	—	—	—	p
Potamilla reniformis	—	—	—	—	—	—	—	—	—	—	—	p	—
Dasychone bombyx	—	—	—	—	—	—	—	—	—	—	c	—	p
Sabellidae: unidentified	—	—	—	—	—	—	—	—	—	—	—	—	p
Serpula vermicularis	—	—	p	—	—	—	p	p	p	—	p	—	—
Hydroides norvegica	—	p	—	—	p	p	p	—	p	—	c	—	—
Pomatoceros triqueter	p	p	p	p	c	p	—	—	—	—	a	p	—
Protula tubularia	p	—	—	—	—	—	—	—	—	—	—	—	—
Filograna implexa	—	—	p	—	—	—	—	—	—	—	p	—	—
Spirorbis sp.	—	—	—	p	—	—	p	—	—	—	—	p	—

p = present c = common a = abundant.

TABLE 7. POLYCHAETA BORING OR INHABITING CREVICES IN EMPTY SHELLS OR SERPULID TUBES (RELATIVE ABUNDANCE)

Shell ...	*Ostrea*			*Modiolus*	*Glycimeris*		Serpulid tubes				
Station number ...	*40*	*54*	*54*	*52*	*53*	*46*	*68*	*34*	*48*	*46*	*69*
Depth (fathoms) ...	16	21	21	24	21	24	10	15	21	24	25
Species											
Pholoë minuta	—	p	—	—	—	—	—	p	—	—	—
Eulalia viridis	—	p	—	—	—	—	—	—	—	—	—
E. viridis var. *aurea*	—	—	—	—	—	—	—	—	—	p	—
E. bilineata	—	p	p	—	—	—	—	—	—	—	—
E. sanguinea	—	—	—	—	—	—	—	p	—	—	—
E. fucescens	—	—	p	—	—	—	—	—	—	—	—
E. pusilla	p	—	p	—	—	—	—	—	—	—	—
E. macroceros	p	—	—	—	—	—	—	—	—	—	—
Notophyllum foliosum	—	p	—	—	—	p	—	—	p	—	—
Castalia punctata	—	p	—	—	—	—	—	—	—	—	—
Syllis armillaris	—	c	c	—	—	—	p	—	—	—	p
S. amica	—	—	—	—	—	—	p	—	—	—	—
S. variegata	—	p	p	—	—	—	p	—	—	—	—
Trypanosyllis coeliaca	p	—	—	—	—	—	—	—	—	—	—
Odontosyllis fulgurans	—	—	—	—	—	—	—	p	—	—	—
Eusyllis blomstrandi	—	p	p	—	—	—	p	—	—	—	—
Exogone gemmifera	—	p	—	—	p	—	—	—	—	—	—
Autolytus rubropunctatus	—	p	p	—	—	—	p	—	—	—	—
A. longeferiens	—	p	—	—	—	—	—	—	—	—	—
A. aurantiacus	—	c	p	—	—	—	p	—	—	—	—
Nereis zonata	—	p	—	—	—	—	—	—	—	—	—
Nematonereis unicornis	p	p	—	p	p	p	—	—	—	—	—
Polydora ciliata	—	—	—	—	—	—	p	—	—	—	—
P. caeca	—	p	p	—	—	p	—	—	c	—	—
P. flava	—	—	p	—	—	—	—	c	—	—	—
P. giardi	—	—	—	p	—	—	—	—	—	—	—
Heterocirrus zetlandicus	—	p	p	—	—	—	—	p	—	—	—
Dodecaceria concharum	—	—	—	—	—	—	p	—	—	—	p
Lumbriclymene minor	—	—	c	—	—	c	—	—	—	—	—
Polycirrus denticulatus	p	p	p	—	—	—	—	—	—	—	—
Potamilla torelli	—	—	—	—	—	—	p	—	—	—	—
P. reniformis	—	p	c	—	p	p	—	—	—	—	p

p = present c = common.

TABLE 8. POLYCHAET FAUNA OF HYDROIDS

(Relative abundance.)

Station number ...	*47*	*48*	*49*	*53*	*52*	*46*
Depth (fathoms) ...	20	21	21	21	24	24
Species						
Lepidonotus squamatus	p	—	—	p	p	—
Harmothoë impar	—	—	—	p	p	—
Halosydna gelatinosa	—	—	—	p	—	—
Lagisca extenuata	—	—	p	—	p	p
Eulalia bilineata	p	—	—	p	—	p
E. fucescens	—	—	—	p	—	—
E. pusilla	—	—	—	p	—	—
Notophyllum foliosum	—	—	—	—	—	p
Mystides limbata	—	—	—	—	—	p
Castalia punctata	p	—	—	p	p	p
Syllis armillaris	a	p	p	a	—	p
S. variegata	p	p	—	—	—	—

TABLE 8 (*continued*)

Station number ...	*47*	*48*	*49*	*53*	*52*	*46*
Eusyllis blomstrandi	p	c	c	c	c	a
Trypanosyllis zebra	—	—	—	p	—	—
Exogone gemmifera	—	—	—	c	p	p
Autolytus pictus	—	—	—	p	—	—
A. rubropunctatus	p	—	—	p	—	—
A. aurantiacus	p	c	—	p	c	—
A. longeferiens	—	a	—	p	a	c
A. prolifer	—	—	—	—	p	—
Autolytus sp.	—	—	—	p	—	—
Procerastea perrieri	—	—	—	—	p	—
Nereis pelagica	—	—	—	p	p	—
N. zonata	—	—	—	p	—	—
Ephesia peripatus	p	—	—	—	p	—
Glycera lapidum	—	—	—	—	p	—
Lumbriconereis fragilis	—	p	—	—	—	—
Polydora caeca	—	—	—	p	—	—
Melinna cristata	—	—	—	—	p	—
Pista maculata	—	—	—	—	p	—
Nicolea zostericola	—	—	—	—	—	p
Polymnia nebulosa	—	—	—	—	p	—
Polycirrus denticulatus	—	—	p	p	p	p
Trichobranchus glacialis	—	p	—	—	—	—
Hydroides norvegica	—	p	—	—	c	c
Spirorbis spirillum	—	a	—	—	a	c

a = abundant c = common p = present.

TABLE 9. POLYCHAET FAUNA OF CLEAN SAND

(Number per square metre or relative abundance.)

Station number ...	*65*	*59*	*31*
Depth (fathoms) ...	2–5	3–4	10
Dredge or grab ...	D	G	D
Number of samples ...	1	5	1
Species			
Harmothoë imbricata	p	—	—
Sthenelais limicola	c	—	—
Eteone foliosa	—	2	—
E. longa	—	4	—
Mystides limbata	—	2	—
Kefersteinia cirrata	p	—	—
Exogone verugera	—	4	—
Nereis pelagica	p	—	—
Platynereis dumerilii	p	—	—
Nephthys cirrosa	p	—	p
Glycera convoluta	—	6	—
G. gigantea	—	2	—
G. rouxi	—	2	—
Goniada maculata	—	2	—
Eone nordmanni	—	2	—
Nematonereis unicornis	—	2	—
Scoloplos armiger	—	58	p
Spiophanes bombyx	—	6	—
Polydora caulleryi	—	2	—
Magelona papillicornis	—	2	—
Chaetozone setosa	—	10	—
Heterocirrus zetlandicus	—	—	p
Capitella capitata	—	8	—
Clymene oerstedii	—	10	—
Owenia fusiformis	—	2	—
Pista cristata	c	2	—
Polycirrus denticulatus	p	—	—

c = common p = present.

TABLE 10. COMPOSITION OF THE MACROFAUNA OF VARIOUS DEPOSITS

Habitat	Percentage of total population per station					Polychaeta				
						No. of specimens per m²		No. of species per station		
	Polychaeta Mean	Crustacea Mean	Mollusca Mean	Echinodermata Mean	Others Mean	Range	Mean	Range	Mean	Total species
Mud	62	15·3	9·7	4·3	8·7	26–109	56	4–12	7·9	27
Muddy sand, no shell	43·6	7·3	31·5	6·5	11·1	66–574	174	8–31	19·3	64
Muddy sand, with shell	43·3	15·5	13	11·9	16·3	95–368	221	14–50	31·6	122
Fine shell gravel	64	5·2	9·2	11·4	10·2	150–466	342	21–39	29	85
Fine stony gravel	49	17·7	7·7	16	9·6	—	—	6–45	24	66
Coralline gravel	58	8·4	7	22·1	4·5	180–292	244	18–31	27	58
Coarse shell gravel	—	—	—	—	—	—	—	2–13	—	27
Large stones	—	—	—	—	—	—	—	10–24	18	41
Clean sand	60	21·5	17·5	1	—	120	—	3–19	—	27
Boring in shells	—	—	—	—	—	—	—	1–19	6·4	32
Sponges	—	—	—	—	—	—	—	6	—	6
Hydroids	—	—	—	—	—	—	—	4–20	11·5	37

TABLE 11. STATION LIST

No.	Date	Position	Depth (fm.)	Bottom deposit	Gear used	No. of hauls
1	16. ii. 53	1½ miles W. of Niarbyl	15	c.gr. & m.s.	V-V.G.	5
2	16. ii. 53	4 miles W. of Niarbyl	20	m.s. & sh.gr.	V-V.G.	5
3	16. ii. 53	5 miles W. of Niarbyl	25	m.s. & sh.gr.	V-V.G.	5
4	16. ii. 53	5½ miles W. of Niarbyl	30	m.s.	V-V.G.	5
5	9. iii. 53	1½ miles W. of Niarbyl	15	c.gr. & m.s.	V-V.G.	5
6	9. iii. 53	6½ miles W. of Niarbyl	35	m.s.	V-V.G.	5
7	9. iii. 53	7 miles W. of Niarbyl	40	m.	V-V.G.	5
8	9. iii. 53	8½ miles W. of Niarbyl	45	m.	V-V.G.	5
9	9. iii. 53	10½ miles W. of Niarbyl	50	m.	V-V.G.	5
10	20. iv. 53	½ mile N. of Fleshwick Bay	9	f.st.gr.	S.N.D.	1
11	20. iv. 53	Fleshwick Bay	10	f.st.gr.	S.N.D.	1
12	18. iv. 53	1½ miles S. 80° W. of Niarbyl	15	c.gr. & m.s.	V-V.G.	5
13	18. iv. 53	4 miles S. 80° W. of Niarbyl	20	m.s. & sh.gr.	V-V.G.	5
14	18. iv. 53	5½ miles S. 80° W. of Niarbyl	30	m.s. & sh.gr.	V-V.G.	5
15	18. iv. 53	9 miles S. 80° W. of Niarbyl	40	m.	V-V.G.	5
16	18. iv. 53	10 miles S. 80° W. of Niarbyl	45	m.	V-V.G.	5
17	27. viii. 53	12 miles S. 80° W. of Peel	50	m.	V-V.G.	5
18	27. viii. 53	13 miles N.W. of the Sound	55	m.	V-V.G.	5
19	27. viii. 53	6 miles N. 55° W. of the Sound	30	m.s.	V-V.G.	5
20	14. x. 53	3 miles W. of Bradda Head	20	sh.s. & sh.gr.	V-V.G.	3
21	14. x. 53	4½ miles W. of Sloc	20	sh.s. & sh.gr.	V-V.G.	3
22	14. x. 53	4 miles W. of Niarbyl	20	m.s. & sh.gr.	V-V.G.	4
23	14. x. 53	5 miles W. of Niarbyl	25	m.s. & sh.gr.	V-V.G.	5
24	14. x. 53	5½ miles W. of Niarbyl	30	m.s.	V-V.G.	5
25	14. x. 53	5½ miles W. of Sloc	25	m.s. & sh.gr.	V-V.G.	5
26	15. x. 53	5 miles W. of Bradda Head	25	sh.s. & sh.gr.	V-V.G.	5
27	15. x. 53	6 miles W. of Bradda Head	30	sh.s. & sh.gr.	V-.VG.	3
28	15. x. 53	9 miles W. of Bradda Head	29	m.s. & sh.gr.	V-V.G.	3
29	15. x. 53	10 miles W. of Bradda Head	40	m.s. & *Turr*.sh.	V-V.G.	3
30	24. xi. 53	1½ miles W. of Niarbyl	15	c.gr. & m.s.	S.N.D.	1
31	24. xi. 53	½ mile W. of Niarbyl	10	s.	S.N.D.	1
32	24. xi. 53	½ mile S.S.W. of Niarbyl	10	coarse st.gr.	S.N.D.	1
33	9. ii. 54	1 mile W. of Bradda Head	17	m.s. & sh.gr.	S.N.D.	1
34	9. ii. 54	2 miles W. of Sloc	15	large stones, f.st.gr. & m.s.	S.N.D.	1
35	9. ii. 54	3 miles W. of Sloc	18	m.s. & sh.gr.	S.N.D.	1
36	9. ii. 54	9½ miles W. of Sloc	40	m.s. & *Turr*.sh.	V-V.G.	3
37	9. ii. 54	11 miles W. of Sloc	50	m.	V-V.G.	3
38	9. ii. 54	7 miles W. of Sloc	30	m.s. & sh.gr.	V-V.G.	3
39	11. iii. 54	Off P.E. breakwater	5	f.st.gr.	N.D.	1
40	11. iii. 54	¼ mile W. of Charran	16	st.gr.	N.D.	1
41	1. iv. 54	8 miles S. 80° W. of Chicken R.	35	m.s. & *Turr*.sh.	V-V.G.	3
42	1. iv. 54	10 miles S. 80° W. of Chicken R.	38	m.	V-V.G.	3
43	1. iv. 54	12 miles S. 80° W. of Chicken R.	35	m.	V-V.G.	3
44	1. iv. 54	6 miles S. 80° W. of Chicken R.	34	m.s. & sh.gr.	S.N.D.	1
45	1. iv. 54	4 miles S. 80° W. of Chicken R.	32	m.s. & sh.gr.	S.N.D.	1
46	1. iv. 54	2 miles S. 80° W. of Chicken R.	24	shells & sh.gr.	4 ft. D.	1
47	19. iv. 54	1½ miles S. 10° W. of Scarlet P.	20	stones	4 ft. D.	1
48	19. iv. 54	3 miles S. 60° W. of Langness	21	shells & stones	4 ft. D.	1
49	19. iv. 54	2¾ miles S. 15° W. of P. St. M.	21	shells	4 ft. D.	1
50	19. iv. 54	1 mile S.E. of Chicken R.	25–30	stones	4 ft. D.	1
51	19. iv. 54	2 miles S. 22° W. of Chicken R.	24	sh.gr. & stones	4 ft. D.	1
52	19. iv. 54	2 miles N. 80° W. of Chicken R.	25	sh.gr. & shells	4 ft. D.	1
53	19. iv. 54	2¾ miles S. 80° W. of Bradda Head	21	shells	4 ft. D.	1
54	19. iv. 54	3½ miles S. 80° W. of Bradda Head	21–24	shells	4 ft. D.	1
55	26. iv. 54	11 miles N. 40° W. of the Sound	53	m.	V-V.G.	2
56	26. iv. 54	13 miles N. 40° W. of the Sound	58	m.	V-V.G.	2
57	26. iv. 54	14 miles N. 40° W. of the Sound	65	m.	V-V.G.	2
58	26. iv. 54	15 miles N. 40° W. of the Sound	70	m.	V-V.G.	2

TABLE 11 (*continued*)

No.	Date	Position	Depth (fm.)	Bottom deposit	Gear used	No. of hauls
59	26. iv. 54	Port Erin Bay	3–4	s. & f.st.gr.	V-V.G.	5
60	12. v. 54	6 miles N. 60° W. of the Sound	31	m.s. & sh.gr.	V-V.G.	3
61	12. v. 54	9 miles N. 60° W. of the Sound	41	m.s. & *Turr*.sh.	V-V.G.	3
62	12. v. 54	10½ miles N. 60° W. of the Sound	50	m.	V-V.G.	3
63	12. v. 54	15 miles N. 60° W. of the Sound	60	m.	V-V.G.	3
64	12. v. 54	7½ miles N.W. of the Sound	37	m.s.	V-V.G.	5
65	13. xi. 53	Port Erin Bay	2–3	s.	N.D.	2
66	20. i. 54	½ mile N. of the Sound	15	*Chlamys opercularis*	4 ft. D.	1
67	14. x. 52	¼ mile W. of Bradda Head	12	shells & sh.gr.	4 ft. D.	2
68	24. x. 52	Bay Fine	12	shells	4 ft. D.	1
69	5. xi. 52	5 miles S. of P. St. M.	27	*Modiolus* epif.	4 ft. D.	1
70	11. xi. 52	1 mile S. of Spanish Head	17	sh.gr. & m.s.	S.N.D.	1
71	11. xi. 52	1 mile N.N.W. of Bradda Head	18½	sh.gr. & m.s.	S.N.D.	1
72	11. xi. 52	2 miles S.W. of Chicken R.	26	shells & sh.gr.	S.N.D.	1
73	28. vii. 53	Bay Fine	15	*Chlamys opercularis*	4 ft. D.	1
74	6. x. 53	Off P. E. breakwater	10	rock	Trawl	
75	1. xii. 52	Fleshwick Bay	6	f.st.gr.	S.N.D.	1
76	30. ix. 52	7 miles N. 30° W. of the Sound	35	m.s.	V-V.G.	10
77	17. xi. 52	7 miles N. 30° W. of the Sound	35	m.s.	V-V.G.	10
78	23. xii. 52	7 miles N. 30° W. of the Sound	35	m.s.	V-V.G.	10

Abbreviations

Gear used: V-V.G., van Veen bottom sampler ($1/10\ m^2$); N.D., naturalist's dredge (2 ft. 6 in.); S.N.D., small naturalist's dredge (1 ft. 6 in.); 4 ft. D., 4 ft. scallop dredge.

Bottom deposit or contents of haul: c.gr., coralline gravel; f.st.gr., fine stony gravel; m., mud; m.s., muddy sand; s., sand; sh.s., shell sand; sh.gr., shell gravel; *Turr*.sh., empty shells of *Turritella communis*.

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THE OCCURRENCE AT PLYMOUTH OF *DICTYOCOTYLE COELIACA* NYBELIN, 1941 (TREMATODA: MONOGENEA)

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(Plate I)

Dictyocotyle coeliaca is a somewhat unusual monogenetic trematode in that it is endoparasitic and not ectoparasitic, as are most monogeneans. It was first described by Nybelin (1941) from two specimens, one of which had been collected by Swenander in 1905 from the coelom of *Raia radiata* caught at Trondhjem, and the other collected by Nybelin himself from *R. lintea* caught at Göteborg. Later, according to Sproston (1946), Mr Quinten Geering at Cambridge found three trematodes firmly attached to the liver of a young *Raia* sp. from Plymouth. These parasites were deposited in the British Museum, where they were provisionally identified as damaged specimens of *Calicotyle krøyeri*, a common parasite of the cloaca of various species of *Raia*. However, these specimens were subsequently examined by Dr Brinkmann (1952) of Bergen, and he, having seen the type material, was able to identify the Plymouth specimens as *Dictyocotyle coeliaca*.

Two parasites collected in the Zoology Department at Sheffield University from the outside of the stomach of *Raia clavata*, believed to have been landed at Brixham, were identified by Dawes (1948) as Nybelin's species, but Dawes regarded the species as belonging to the genus *Calicotyle*.

Hunter & Kille (1950) examined rays brought from Scottish fishing grounds into the Zoology Department at Edinburgh University, and found *Dictyocotyle coeliaca* in sixteen of fifty-five specimens of *Raia naevus* and in thirty-one of seventy-three specimens of *R. radiata*, but none in *R. batis*, *R. montagui*, *R. brachyura*, *R. clavata* and *R. fullonica*. These authors gave a description of *Dictyocotyle coeliaca* and compared the parasite with *Calicotyle krøyeri*.

In the present observations, examinations have been made of specimens of the four species of *Raia* commonly landed at Plymouth, in the period May 1955 to August 1956 inclusive. The coelom and viscera of each host specimen were searched for the presence of *Dictyocotyle coeliaca*, and the results are included in Table 1.

Of the thirty-five infected specimens of *Raia naevus*, nineteen were males and sixteen were females. The mean infestation of parasites per fish was 3·97, and the maximum number of parasites collected from a single host specimen was twenty-two. There appeared to be no particular site of infestation within the coelom, and living specimens of the trematode were collected, at periods varying from 1 to 18 hr after the death of the host, from all the inner surfaces of the body wall, and less frequently, from the viscera.

TABLE 1

Host	No. of specimens examined	No. infected
Raia naevus	135	35
R. clavata	478	0
R. montagui (= *R. maculata*)	420	0
R. brachyura	34	0

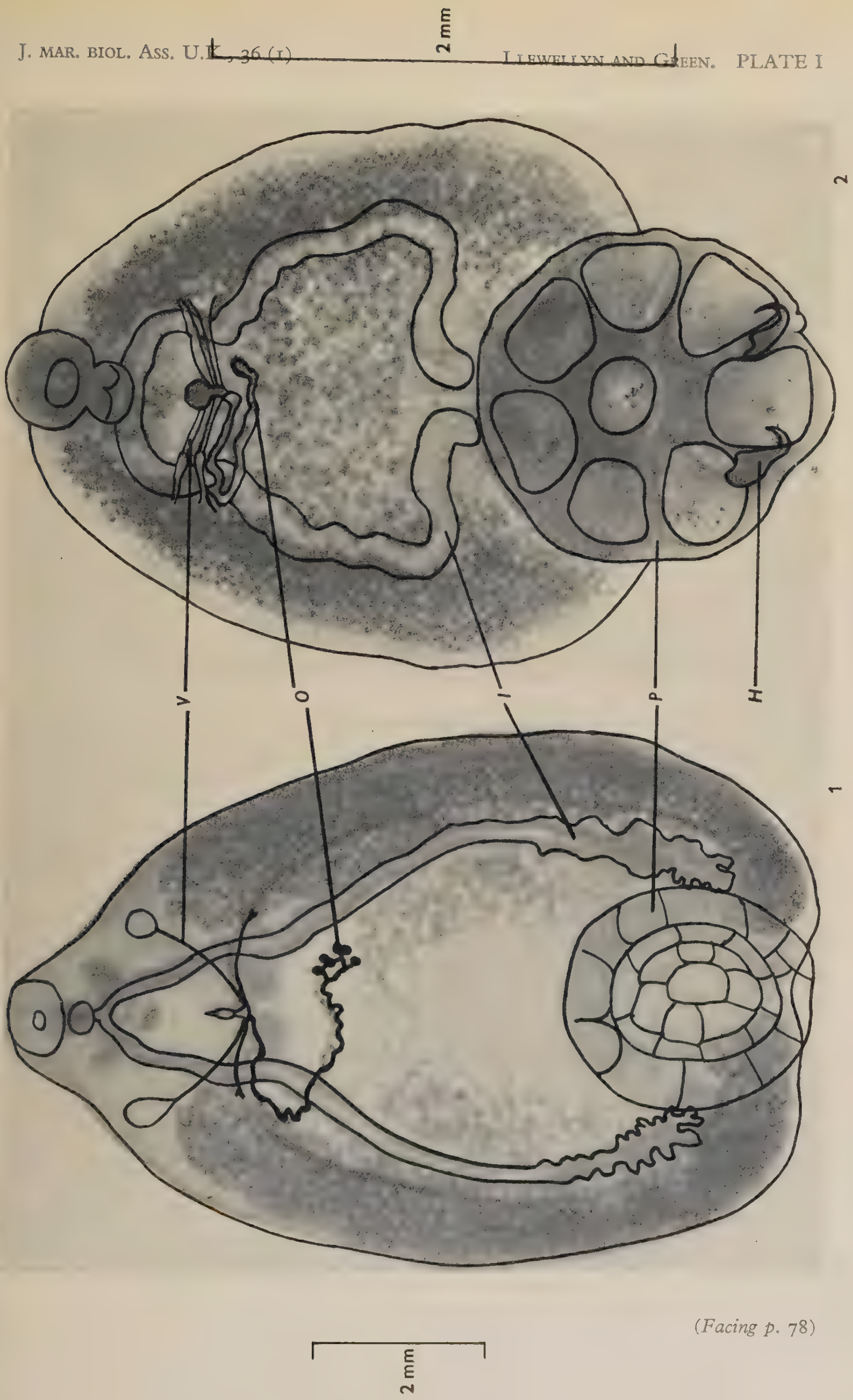
On several occasions specimens of both *Dictyocotyle coeliaca* and *Calicotyle krøyeri* have been found in the same individual *Raia naevus*, the former in the coelom and the latter in the cloaca. Photographs illustrating the differences between the two parasites, previously noted by Hunter & Kille, are included in Pl. I, figs. 1 and 2.

It may be concluded then that *Dictyocotyle coeliaca* is at least as common in *Raia naevus* at Plymouth (about 26% infestation) as are most other monogeneans on their respective hosts (see table of frequencies in Llewellyn, 1956); the remarkable feature is that it has escaped attention, since *R. naevus*, along with other rays, is frequently supplied to University Departments for class dissection.

In view of the absolute restriction of *Dictyocotyle coeliaca* to *Raia naevus* in over 1000 *Raia* specimens examined at Plymouth in the present study, and to *R. naevus* and *R. radiata* in Hunter & Kille's (1950) study of 181 specimens of rays from Scottish waters, it seems distinctly possible that the Brixham host ('*Raia clavata*') of the material sent to Dawes (1948) had been mis-identified.

SUMMARY

In a sample of over 1000 rays belonging to four species of *Raia* examined at Plymouth between May 1955 and August 1956, the 'very rare' monogenetic trematode *Dictyocotyle coeliaca* Nybelin, 1941 (total of four previous records) was found in the coelom of thirty-five out of 135 specimens of *Raia naevus*, but absent from 478 *R. clavata*, 420 *R. montagui* and 34 *R. brachyura*.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(Facing p. 78)

REFERENCES

Brinkmann, A., 1952. Fish trematodes from Norwegian Waters. *Univ. Bergen Årb. naturv. R.*, No. 1.

Dawes, B., 1948. A rare coelom-dwelling trematode. *Nature, Lond.*, Vol. 161, p. 642.

Hunter, G. C. & Kille, R. A., 1950. Some observations on *Dictyocotyle coeliaca* Nybelin, 1941. *J. Helminth.*, Vol. 24, pp. 15–22.

Llewellyn, J., 1956. The host specificity, micro-ecology, adhesive attitudes, and comparative morphology of some trematode gill parasites. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 113–27.

Nybelin, O., 1941. *Dictyocotyle coeliaca* n.g. n.sp. Ein leibeshöhlebewohnender monogenetischer Trematode. *Göteborgs VetenskSam. Handl.*, Följ. vi, Ser. B, Bd 1, No. 3, 19 pp.

Sproston, N. G., 1946. A synopsis of the monogenetic trematodes. *Trans. zool. Soc. Lond.*, Vol. 25, pp. 185–600.

EXPLANATION OF PLATE I

A comparison of the morphology of *Dictyocotyle coeliaca* Nybelin, 1941 with that of *Calicotyle krøyeri* Diesing, 1850. Fig. 1. *Dictyocotyle coeliaca.* Fig. 2. *Calicotyle krøyeri. I*, posterior ends of intestinal caeca of *Dictyocotyle* sacculated and end well behind anterior edge of posterior adhesive organ; not sacculated in *Calicotyle*, and end in front of anterior edge of posterior adhesive organ. *H*, hooks present in *Calicotyle*, absent in *Dictyocotyle*. *O*, proximal region of ovary 5–7 lobed in *Dictyocotyle*, simple in *Calicotyle*. *P*, posterior adhesive organ of *Dictyocotyle* with numerous irregular shallow loculi of various sizes; of *Calicotyle* with a central stalk and seven peripheral loculi all of similar shape and size; posterior adhesive organ in *Dictyocotyle* reaches posterior border of body, in *Calicotyle* it projects well beyond the posterior margin of the body. *V*, vaginae pass obliquely anteriorly to open in front of vitellaria in *Dictyocotyle*, but pass transversely in *Calicotyle*.

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THE FEEDING MECHANISM AND STRUCTURE OF THE GUT OF *OWENIA FUSIFORMIS* DELLE CHIAJE

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(Text-figs. 1 and 2)

In 1901 Arnold Watson published a paper on the habits of *Owenia fusiformis* delle Chiaje (= *Ammochares filiformis* Watson) in his now classic series of papers on the tube-building habits of various polychaetes. There is little to be added to his description in this respect, but Watson was not concerned with the feeding mechanism, which receives but brief mention. *O. fusiformis* has previously been studied by a number of workers, but none has given much attention to the morphology of the gut or to the method of feeding which are the main considerations of the present paper.

O. fusiformis has been studied from other aspects in some detail, notably by von Drasche (1885), who, while dealing little with the gut, did note that the mid-gut region has a green colour. Claparède (1868, 1873) and Saint-Joseph (1898) also mention this. Some account of other features of the morphology of *Owenia* will be found in the papers of Gilson (1893, 1897), Lo Bianco (1893), Eisig (1887) and McIntosh (1915), while the development and metamorphosis of the mitraria larva belonging to this species is described in the well-known paper of Wilson (1932).

O. fusiformis is not uncommon in clean sand below low water of neap tides on British shores, often in beaches subject to some disturbance and not tenable by polychaetes with a permanently fixed tube or burrow. Though local in occurrence, the species is cosmopolitan in distribution. The worms studied in the present work were collected from Tor Abbey sands, Torquay, Devon, and from near St Mawes in Cornwall. Elwes (1910) first reported *Owenia* as the commonest polychaete in Tor Abbey sands; Wilson collected worms from this beach for his developmental studies (1932), and it is certainly still numerous there. The worms construct long flexible tubes up to 15–20 cm long and 2–5 mm across, consisting of flat grains arranged like roof tiles with the free edge upwards; white or lightly coloured grains seem to be preferred, even when relatively scarce, so that the tubes are often conspicuous, as in the reddish sand of Torquay. The flexibility and probably also the arrangement of the sand grains enables the worm to work the tube up and down in the sand and to rebury itself when necessary. Worms can withdraw the tube completely beneath the surface, but when feeding in the laboratory the tube may project 2–3 cm above the sand. Possibly the tubes do not project as much as this in nature. *Owenia* is very hardy, as Watson (1901) found, and will live in the laboratory for long periods without aeration.

STRUCTURE OF THE CROWN AND THE FEEDING MECHANISM

The tip of the tube forms an elastic cone of transparent material which almost closes when the worm retracts; during feeding the crown and the most anterior segments are pushed through the minute opening at the tip of the cone which is then distended, and worms when undisturbed may remain for long periods in this position. On other occasions the crown may be seen within the cone, but it sems unlikely that feeding takes place to any significant extent at this time, though some respiratory exchange may continue. The cone is probably of great protective value when the worm is moving within the tube through the sand. The crown has an elaborate blood supply, and is light red, brown or greenish in colour; while there is no doubt that it is respiratory in function, it also participates in feeding.

The crown is a bilaterally symmetrical, branched structure developed from the prostomium, and has some mobility. Though short it is twice the diameter of the body when expanded (Fig. 1). There are four main branches or units on each side (Fig. 1 A), and each unit (Fig. 1 B) is divided into 4–6 branches, each of which ends in 2–4 small bifid lobes (Fig. 1 C). While there is some variation in arrangement, these lobes are almost always arranged in pairs, strongly ciliated on their oral surface, with the stem of each pair raised at the edges to form a gutter (Fig. 1 C). There are long cilia on these edges which beat inwards towards the axis of the stem, and shorter cilia in the gutter itself which beat towards the mouth. The long cilia on the edges of the gutter cause swirling movements between adjacent branches sweeping particles into the crown. Particles which are too large to be ingested usually do not come to rest in the gutter at all, or, if arrested, collect along the edges and are eventually cleaned off by muscular movements, either by convulsive movements of the whole crown, or by more local movements. Cilia are entirely absent from the aboral surface of the crown, but mucus gland cells are distributed over both oral and aboral surfaces.

The crown forms a funnel at the base closed by three lips, a single dorsal lip, and two ventro-lateral in position. These lips are all expansions of the prostomium, and when dilated close the mouth by coming together at the centre (Fig. 1 A). They are hollow and extremely mobile; dilation is caused by the inflow of coelomic fluid, but each lip has a complicated musculature and is capable of a variety of rippling movements. The crown contains an extensive coelomic cavity. Closure of the crown is brought about by the circular crown muscles acting against the hydrostatic pressure of the body fluid; when the crown is expanded these crown muscles are relaxed (Fig. 1 B). These circular muscles extend into all the branches except into the bifid tips, so that each gutter is capable of a certain amount of inrolling which can assist both in directing the strings of particles towards the mouth, and in freeing the crown of unwanted matter. While convulsive closure of the whole crown takes place

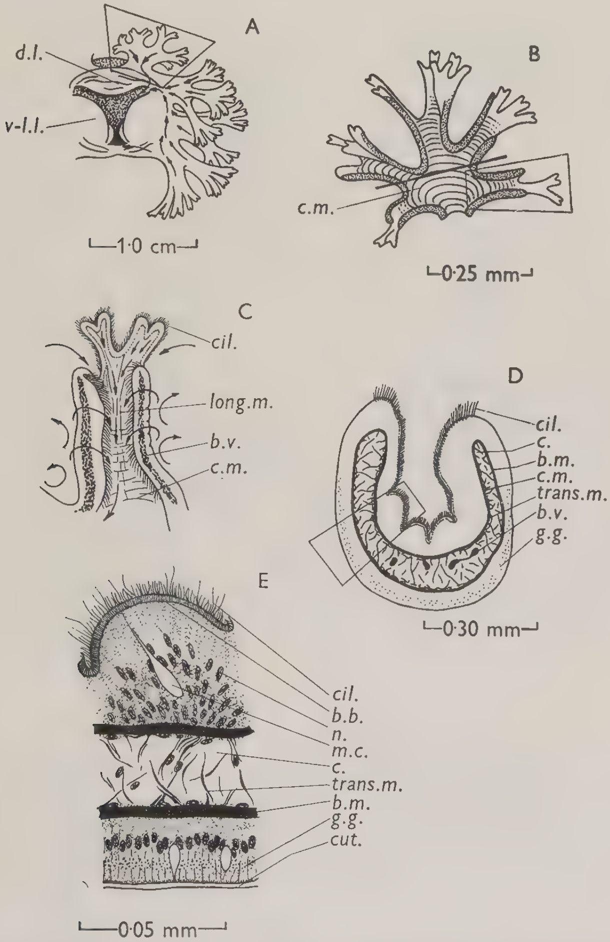

Fig. 1. The crown and feeding mechanism. A, the left half of the crown seen from above; arrows indicate direction of ciliary currents; the area marked is shown in B. B, a single unit showing the gutter and circular muscles; the area marked is shown in C. C, a single lobe showing arrangement of cilia and direction of ciliary currents. D, section through a unit corresponding to the plane of the line shown in A; the area marked is shown in E. E, section through the axis of a unit. *b.b.* brush-border; *b.m.* basement membrane; *b.v.* blood vessel; *c.* coelom; *c.m.* circular muscle; *cil.* cilia; *cut.* cuticle; *d.l.* dorsal lip; *g.g.* granules of green pigment; *long.m.* longitudinal muscle; *m.c.* mucus gland cell; *n.* nuclei; *trans.m.* transverse muscle; *v-l.l.* ventro-lateral lip.

at intervals during feeding, different parts of the crown are capable of independent movement. There are also muscle fibres across the coelomic space between aboral and oral surfaces (Fig. 1 D, E) and longitudinal fibres in the bifid tips (Fig. 1 C).

Ciliary feeding, which is continued with the worm in a more or less erect posture may continue for many hours. During this time the lips may undergo rippling movements which assist the food strings into the mouth, but they participate little in this type of feeding and they are better adapted for manipulating sand grains and detritus which are also ingested at other times.

The ingestion of sand grains is brought about by the animal bending over, usually within the tube, until the crown sweeps the surface of the sand. The crown itself may then close in order to pick up a number of grains, which are then manipulated by the three lips already mentioned. If the grains are about 0·2 mm or less in length they are ingested; if larger, rejected or used for building or repairing the tube. The buccal organ, which is a double-lipped structure lying mid-ventrally between and beneath the ventro-lateral lips, usually remains completely concealed during feeding. The buccal organ is adapted for applying the sand grains to the tube, as Watson (1901) described, and does not participate in the feeding process. It is this buccal organ which is homologous with the buccal organs, bulbs or ventral lip-folds in other sedentary polychaetes, and it is interesting that in *Owenia* it has become adapted solely for tube-building and is not used at all in feeding.

Owenia can thus feed in two distinct ways, either in a ciliary manner, or by taking up much larger particles with the lips. The deeper-water genus *Myriochele*, the only genus other than *Owenia* included in the family, has a simple unbranched crown in the form of a hollow cone, and it seems likely that this worm feeds almost entirely on detritus in a similar way.

MORPHOLOGY OF THE GUT

Most previous writers have described the gut as a simple tube leading from mouth to anus. This is a rather simplified description, as even in external view the gut is divisible into three parts, which histologically are abruptly separated. Some idea of the extent and composition of these parts may be obtained from Fig. 2 A–D.

A short oesophagus leads from the mouth to a yellow tube with thick and much involuted walls. Farther back the gut assumes a deep green colour, and this region has somewhat thinner walls and is less infolded; between the level of the 5th and 6th pairs of tori the green colour diminishes, and the hind gut is very thin-walled and capable of great distension.

The floor of the crown has ciliated tracts leading down into the mouth as already described. The whole of this buccal region is ciliated as is the oesophagus, the tall epithelial cells of which are characterized by a brush-border, long cilia, finely granular cytoplasm and elongate nuclei (Fig. 2E). Basal granules beneath the brush-border and converging fibres characteristic of cells of this kind may be seen leading from the granules towards the middle of the cell where the nucleus lies. Gland cells, staining blue with Azan, occur in the epithelium of the buccal region and in the oesophagus, between the

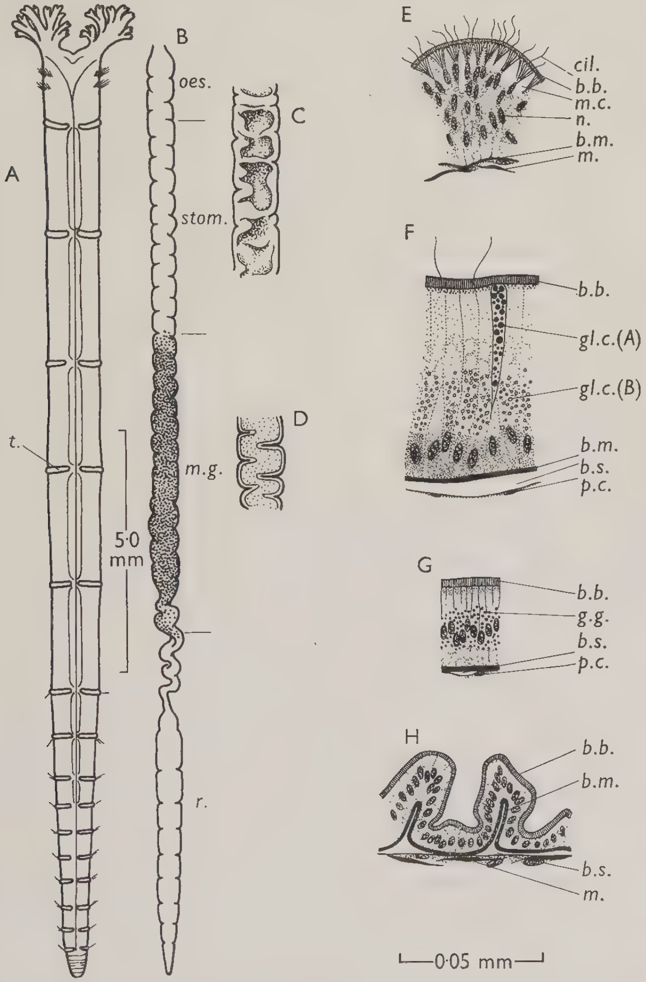

Fig. 2. The morphology and histology of the gut. A, the whole worm in ventral view. B, external appearance of the whole gut; anterior part of rectum is empty, the hinder part filled. C, part of the stomach showing thick walls and involutions. D, part of the mid-gut showing the thin walls and simpler infoldings. E–H, transverse sections of the oesophagus (E), stomach (F), mid-gut (G) and rectum (H), all drawn to the same scale. Abbreviations not used in Fig. 1: *b.s.* blood sinus; *gl.c.(A)*, gland-cell with granules with azocarmine affinity; *gl.c.(B)*, with granules with aniline-blue affinity; *m*, muscle; *m.g.* mid-gut; *oes.* oesophagus; *p.c.* peritoneal cell; *r.* rectum; *stom.* stomach; *t.* torus.

ciliated cells. These mucus cells are scattered throughout the length of the oesophagus which extends back to the level of the 1st pair of tori; externally the oesophagus is distinguishable by its lighter colour from the yellow stomach, but internally there is a sharp histological transition.

The stomach has much thicker walls than the oesophagus and is much involuted (Fig. 2C). A few ciliated cells extend into the anterior part of the stomach, but cilia are absent from the main part of the stomach and from all the more posterior regions of the gut. The stomach extends back to the level of the 3rd pair of tori. The epithelial cells are mainly glandular as indicated by their heavily staining granular contents. When stained with Azan the outer part of the cell is vacuolated and contains red-staining granules, the middle part has rather larger granules with a much greater affinity for aniline blue, but without vacuoles, and the inner part of the cell contains the nucleus lying beneath these granular zones, within a dense red-staining cytoplasm (Fig. 1F). Other cells, presumably also secretory, contain granules with a strong affinity for azocarmine; these seem to be more numerous posteriorly and are represented in the most posterior part of the stomach where there are no cells with aniline-blue staining granules.

The transition from the stomach to the mid-gut at the level of the 3rd pair of tori is accompanied by another abrupt histological change. The wall of the mid-gut is much thinner than that of the stomach, the cells with blue-staining granules are entirely lacking. The cells of the mid-gut have a well-marked brush-border, nuclei nearer the middle of the cell than in the stomach, and a fine granular cytoplasm (Fig. 2G). The mid-gut is recognizable externally by its deep green colour which is due to granules of pigment in the middle of the cell. The pigment remains even in Bouin-fixed specimens, but some worms were fixed in Helly's fluid and lightly stained in orange G in order to get a better appreciation of their distribution. The granules vary in size between 3 and 27 μ; they seem structureless and are rather irregular in outline. The granules freed from the tissue did not appear to be motile, and there was nothing to indicate that they represented living plants as suggested by Berkeley (1930) for the rather similar green pigment in the mid-gut of *Chaetopterus*. A few granules of a similar green pigment appear in the epidermis of the crown and other parts of the body of some specimens (Fig. 1D). As noted by Watson (1901) the amount present varies greatly; some of the worms from Torquay collected in April were completely green.

The mid-gut passes into a thin-walled distensible rectum at the level of the 5th–6th pairs of tori (Fig. 2H). It is here that sand grains and other matter collect before being defaecated in discrete pellets. Defaecation seems to be an intermittent process, and a great deal of matter may collect in the extensive rectum before the worm reverses in its tube to void the contents. The faecal pellets are surrounded by a thin membrane which stains blue with Azan. There appears to be no mid-ventral gutter so commonly present in this region in other polychaetes, and the rather short cells of the hind-gut appear to have a brush-border without cilia, and with a fine granular cytoplasm. Transmission of the gut contents is presumably mainly muscular, both by the action of the gut musculature itself, though this is very poorly developed in all regions, and by the squeezing action of the body-wall muscles acting through the coelomic fluid. Strong peristaltic waves of contraction may often be seen in the hind part of the body in worms removed from their tubes.

Worms continued feeding when iron saccharate was added to the water, and these were fixed in Bouin's fluid 3 h after the addition. The whole gut of several worms was sectioned, and the sections submitted to the Prussian blue reaction and then lightly counterstained with orange G. Iron particles were found only in the mid-gut cells. It is interesting to note that the region in which the iron had been absorbed coincided exactly with that occupied by the green pigment. No tests have been made for enzymes, but the histology suggests that the stomach is the main, if not the only, secretory part of the gut, the mid-gut the absorptive part, and the rectum serves to store and elaborate the faecal pellets.

ANALYSIS OF THE GREEN PIGMENT

The mid-gut region of over fifty worms was carefully dissected out and extracted in ether: acetic acid, 5:1 mixture, after grinding in a small Griffeth-pattern tissue homogenizer. The dark green solution had a brilliant deep red fluorescence in ultra-violet light. The acetic acid was washed out by shaking repeatedly with distilled water (the first three washings buffered with sodium acetate) until the washings were no longer acid to blue litmus paper. The ethereal solution was then roughly dried by passing through ether-soaked filter paper, and concentrated *in vacuo*. The filtrate was then examined by long-paper chromatography (Kennedy, 1953) using 2:6 lutidine-water system. Two spots were obtained, one with an R_F value of 0·53, suggesting a tetra-carboxyl porphyrin such as coproporphyrin, the other with an R_F value of 0·89, suggesting a mono-carboxyl porphyrin compound such as phaeophorbide.

The ethereal solution was further concentrated *in vacuo* in the cold and extracted with 0·1% w/v HCl, when a red-fluorescent extract was obtained; this further suggested coproporphyrin which has an HCl value of 0·08. The porphyrin was driven back into ether, neutralized with saturated potassium acetate, and the ether solution washed carefully with distilled water, three times. It was then roughly dried by passing through ether-soaked filter paper as before, and evaporated to dryness *in vacuo*. The pigment was then esterified by dissolving the residue in $MeOH:H_2SO_4$, 19:1 mixture. This was left for 48 h at room temperature, after which time the solution was diluted with water and the esterified porphyrin extracted with chloroform. The chloroform extract was washed free from acid with water, roughly dried by filtering through chloroform-soaked paper and redissolved in dry chloroform. The solution was examined with the Hartridge reversion spectroscope and also by the chromatography method of Chu, Green & Chu (1951) when the pigment was found to consist of coproporphyrin III only (Kennedy & Vevers, 1954; Kennedy & Dales, unpublished). The spectrum showed the following maxima (in mμ):

I	II	III	IV
622	566·6	533	500·1

The epiphase from the extract of the ethereal solution with HCl was washed repeatedly with water until free from acid. The solution was examined in the Hartridge reversion spectroscope and also in the 'Unicam' S.P. 600 quartz spectrophotometer when the following maxima characteristic of phaeophorbide-*b* were shown (in mμ):

	I	II	III	IV	Sorêt
Hartridge reversion	652	602	537·5	504·5	
'Unicam'	655	603	537	505	408

The ethereal solution was then extracted with 0·2 % w/v sodium bicarbonate (Willstätter & Stoll, 1913). The hypophases were red-fluorescent, the epiphases were not; this indicates the presence of phaeophorbide-*b* only. There was also a residual yellow pigment in the epiphase after extraction with sodium bicarbonate, which when concentrated had a very slight yellowish fluorescence and which was possibly a carotenoid.

Extracts of green specimens from which the gut had been removed were similarly treated, and the green pigment in the epidermis was also found to be phaeophorbide-*b* alone.

This work was mostly done at Plymouth, and I am indebted to the Director and staff of the Laboratory for their help, and to Dr G. Y. Kennedy of the Department of Cancer Research, Sheffield, in particular, for analysing the green pigment.

SUMMARY

Owenia may feed either by ciliary means or by swallowing sand and detritus. The gut consists of three main parts, a fore-gut or stomach which is secretory, a non-secretory mid-gut region which is absorptive, and a hind-gut which is non-secretory, non-absorptive and serves to elaborate and store the faecal pellets. The mid-gut contains coproporphyrin III and also phaeophorbide-*b*, to which the green colour is due. The green pigment in the epidermis is also phaeophorbide-*b*, but phaeophorbide-*a* is apparently absent from the body.

REFERENCES

BERKELEY, C., 1930. The green bodies of the intestinal wall of certain Chaetopteridae. *Quart. J. micr. Sci.*, Vol. 73, pp. 465–76.

CHU, J. C., GREEN, A. A. & CHU, E. J., 1951. Microchromatography of porphyrin esters. *J. biol. Chem.*, Vol. 190, pp. 643–6.

CLAPARÈDE, É., 1868. Les Annélides Chétopodes du Golfe de Naples. *Mém. Soc. Phys. Genève*, T. 19, pp. 313–584.

—— 1873. *Recherches sur la structure des Annélides Sédentaires.* 199 pp. Geneva: Georg.

DRASCHE, R. VON, 1885. *Beiträge zur feineren Anatomie der Polychaeten. Zweites Heft: Anatomie von* Owenia filiformis *delle Chiaje.* 22 pp. Wien: C. Gerold's Sohn.

EISIG, H., 1887. Monographie der Capitelliden des Golfes von Neapel. *Fauna u. Flora Neapel*, Monogr. 16, 906 pp. Berlin: Friedländer.

ELWES, E. V., 1910. Notes on the littoral Polychaeta of Torquay. Part 3. Other families. *J. mar. biol. Ass. U.K.*, Vol. 9, pp. 59–81.

GILSON, G., 1893. Recherches sur les cellules sécrétantes. II. Les glandes filières de l'*Owenia fusiformis* delle Chiaje. *Cellule*, T. 10, pp. 299–325.

—— 1897. Les valves septales de l'*Owenia. Cellule*, T. 12, pp. 377–416.

KENNEDY, G. Y., 1953. Partition paper chromatography of the prophyrins. *Scand. J. clin. Lab. Invest.*, Vol. 5, pp. 281–4.

KENNEDY, G. Y. & VEVERS, H. G., 1954. The occurrence of porphyrins in certain marine invertebrates. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 663–76.

LO BIANCO, S., 1893. Gli annellidi tubicoli trovati nel Golfo di Napoli. *Atti Accad. Sci. fis. mat. Napoli*, Ser. 2, Vol. 5, pp. 1–97.

McINTOSH, W. C., 1915. *A Monograph of the British Marine Annelids*, Vol. 3, Part 1, 368 pp. London: Ray Society.

SAINT-JOSEPH, A. de, 1898. Les Annélides Polychètes des côtes de France (Manche et Océan). *Ann. Sci. nat.*, Sér. 8, T. 5, pp. 209–464.

WATSON, A. T., 1901. On the structure and habits of the Polychaeta of the family Ammocharidae. *J. Linn. Soc. (Zool.)*, Vol. 28, pp. 230–60.

WILLSTÄTTER, R. & STOLL, A., 1913. *Untersuchungen über Chlorophyll.* 424 pp. Berlin: Springer.

WILSON, D. P., 1932. On the Mitraria larva of *Owenia fusiformis* delle Chiaje. *Phil. Trans.*, B, Vol. 221, pp. 231–334.

J. mar. biol. Ass. U.K. (1957) 36, 91–110
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PRELIMINARY OBSERVATIONS ON THE ROLE OF THE COELOMIC CELLS IN FOOD STORAGE AND TRANSPORT IN CERTAIN POLYCHAETES

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(Text-figs. 1, 2)

INTRODUCTION

To what extent food may be stored, and where such stores may be found in the polychaete body is virtually unknown. There are four main sites where the more detailed economy may take place: in the wall of the gut, in the epidermal layers, in the peritoneum, and in the cells or tissue found within the coelom. The mechanism for the removal of waste products is no better understood than the stages in the utilization and transport of absorbed nutrients. What little information exists is for the most part contradictory and unsupported by quantitative measurement.

While it is hoped to review the function of the chloragocytes in connexion with excretion in a later paper, some discussion of the function of these cells is necessary here in view of their possible relation with food storage and with the identity of the different cells found in the coelomic fluid.

It will be appreciated that most of the work on chloragocytes has been done on oligochaetes, and the identification of these cells in polychaetes has been made by analogy. While there is an extensive literature on coelomic cells (see the bibliographies of Romieu (1923) and Liebman (1946)), the only detailed work on polychaetes is that of Schaeppi (1894) on *Ophelia*, apart from brief descriptions of chloragocytes and general information in the papers of Schneider (1897, 1899) and Picton (1898). There seems to be little agreement about the chemical nature of the chloragosomes or granules occurring within the chloragocytes, and almost any tissue of a yellow or green colour associated with blood vessel or gut has been described as 'chloragogenous' by different writers.

Briefly, there are two main views regarding the function of the chloragocytes and the identity of the chloragosomes. According to one view, the chloragosomes are excretory substances which are liberated by the chloragocytes into the coelomic fluid where they may be taken up by amoebocytes and transported to the nephridia, the gut epithelium, or stored as 'brown bodies'. Supporters of this theory (Cuénot, 1898; Rosa, 1896, 1898; Schneider, 1896, 1899; Willem & Minne, 1900; Kollman, 1908; Romieu, 1923; Abdel-

Fattah, 1955) have described the chloragosomes as consisting of guanin, urates, urea or chitin. Opponents of this view maintain that the chloragosomes represent stored food materials—fat, glycogen, or albumin—and have no relation to excretion. The main exponent of this idea in recent years is Liebman (1946) working on earthworms.

Liebman recognizes two types of cell which he calls *lymphoidocytes* (here called *amoebocytes*) which are small, amoeboid cells, and *trephocytes* which produce, distribute and release nutritive substances into the blood and tissues. Liebman departs from the interpretation of others in identifying the trephocytes with chloragocytes which have become free; in them he traces three stages: (1) cells in which neutral fat is present, (2) those in which phospholipid appears, and (3) cells in which the lipid granules are released into the coelomic fluid or tissues. Liebman infers that the blood in such animals participates little in the transport of nutrients through the body, and that on the contrary it is the 'chloragogue' or trephocyte system which has been elaborated to serve this function. Liebman, however, uses the term 'chloragogue' in a very wide sense to include all the cells covering the gut. He has found in *Eisenia foetida* that wound healing is aided by the aggregation of coelomic trephocytes in the region of the wound, the cells liberating their lipid granules which are then taken up and utilized by the proliferating tissue. Liebman equates the trephocytes with the 'eleocytes' of polychaetes. To what extent this may be justified is discussed later. Issel (1905), working on the enchytraeid *Henlea*, found that the chloragogen covering of the gut was entirely lost at the time of the development of the oocytes, and concluded that this was evidence for the trophic nature of the chloragocytes. Freudweiler (1905) came to the same conclusion in her work on another enchytraeid, *Stercutus*, visualizing the liberation of the chloragosomes (which she also identifies as lipid) into the coelomic fluid with their subsequent uptake by the oocytes.

Those supporting the excretory hypothesis agree that the chloragocytes are modified peritoneal cells in contact with some branch of the intestinal blood system, and that they are not modified amoebocytes (Rosa, 1898; Rice, 1902; Sterling, 1908). The chloragosomes were thought to be guanine by Willem & Minne (1900), who strongly oppose the existence of any fat in the chloragocytes, though both Schneider (1896) and Freudweiler (1905) describe fat in these cells. Cuénot (1898) argued that glycogen, which occurs in large amounts in the peritoneum, is the main storage substance in earthworms, and that the view that the chloragosomes are lipid in nature does not justify the conclusion that they represent important food stores. More recently, Abdel-Fattah (1955) identifies urea in the chloragocytes, and opposes the views of Willem & Minne (1900) in identifying the chloragosomes as guanine. Abdel-Fattah (1955) finds no evidence for the chloragocytes becoming free.

Clearly much of this confusion has resulted from differences in interpretation in the origin and history of the coelomic amoebocytes and trephocytes.

Willem & Minne (1900) regard the chloragocytes as storing the waste products which are later taken up by amoebocytes, and they oppose the view that the chloragocytes themselves become free as Liebman maintains. Romieu (1923), Kollman (1908) and Rosa (1898) derive the trephocytes from amoebocytes, and Romieu identifies in various polychaetes, both fat and excretory substances in these cells.

In polychaetes the increase in abundance of the trephocytes during the early development of the gametes has been noted by a number of observers (Claparède, 1868, 1873; Cuénot, 1891; Romieu, 1921; Fauré-Fremiet, 1929; Herpin, 1921; Dales, 1950), and this implies a nutritive function. None of these writers has identified these trephocytes with chloragocytes or suggested a connexion with excretion.

Some knowledge of the quantities of fat and glycogen in different parts of the body, especially in the trephocytes at different seasons, would aid the elucidation of these problems in polychaetes, and this is the main purpose of the present paper. It is hoped to extend the work in much greater detail.

The species studied were selected for various reasons. Both *Amphitrite johnstoni* Malmgren and *Arenicola marina* (L.) are large and have few septa, so that the collection of the coelomic fluid and contained corpuscles can be reasonably complete. While both species spawn in autumn, *Amphitrite* has a large number of trephocytes at all times, but *Arenicola* has very few. *Nereis diversicolor* O. F. Müller does not lend itself well to this type of study, not only because of its smaller size, but because of the virtual impossibility of collecting the whole coelomic contents owing to the septa. Some determinations of the fats in the body wall and gametes have been made, however, in the light of earlier work (Dales, 1950; Dales & Kennedy, 1954). A few observations have been made on *Terebella lapidaria* (Kahler) for comparison with *Amphitrite*, as the trephocytes differ in containing haemoglobin. The *Amphitrite* were all collected at Noss Mayo, near Plymouth, *Arenicola* from Plymouth and from Chalkwell, Essex, where the *Nereis* were also collected.

Much of this work has been done in the Plymouth Laboratory, and I wish to thank the Director, and Dr L. H. N. Cooper and other members of the staff for help in various ways. In particular I have to thank Mr R. Tozer for collecting *Amphitrite* at times when I could not be at Plymouth, and Dr G. Y. Kennedy for assistance with the analysis of the coelomic cells in *Amphitrite* and *Terebella*. I also wish to thank Mr M. A. Gross for taking the photographs illustrated in Fig. 2.

METHODS

Bloor's methods (1928, 1929) for the analysis of the fat constituents of blood plasma were adapted for the estimation in the different tissues of total fat (estimated as total fatty acids plus sterols) and phospholipid. Cholesterol was estimated by Sperry's method (1938). After some practice total fats could be estimated to an accuracy of ±2% with samples of 50–100 mg of tissue (2–5 mg fat); cholesterol could be estimated rather more accurately; values for phospholipid were found to be less reliable, and have been mostly disregarded. Full details of the procedure adopted in the analysis of the fat are given in the Appendix; the main principles are outlined below.

Extraction was effected with boiling 95% ethanol:ether 3:1 mixture; the extract was made up to a known volume which was then sampled for estimation of total fat, phospholipid, and sterol fractions. For the estimation of total fats, a portion of the extract was saponified, the solvent evaporated off and the pasty residue treated with hot H_2SO_4 to liberate the fatty acids which were then re-extracted with boiling light petroleum. A known quantity of the solution was evaporated to dryness and the fatty acids estimated by oxidation with a chromic acid mixture against a control, followed by back titration with sodium thiosulphate. Phospholipids were estimated by evaporating a portion of the original extract to dryness, re-extracting with light petroleum and then precipitating the phospholipids with acetone and magnesium chloride. The precipitate was washed repeatedly with acetone, and redissolved in ether; a measured quantity evaporated to dryness, and the residue estimated by the chromic acid procedure as before. Total sterols were estimated by treating a portion of the original extract with caustic alkali, and after neutralization, precipitating the sterols as their digitonides. After purification the amount present was estimated by the Liebermann-Burchard reaction, the density of the colour produced being measured in a spectrophotometer against a cholesterol standard at 670 $m\mu$.

For the estimation of glycogen the sample of tissue (about 50 mg) was placed in ethanol at 0° C, or directly in 3 ml. of 30% KOH and refluxed for 2 h. An excess of 95% alcohol was then added to the solution, the tube dipped into a boiling water-bath for 1 min, and then allowed to stand overnight. The precipitate was washed twice in absolute alcohol, and the precipitate repeatedly centrifuged. The walls of the tube were finally washed down with 10 ml. of approximately 0·6 N-HCl and then heated under reflux for 3 h. After cooling, the solution was neutralized with 1·0 N-NaOH against thymol blue, made up to a known volume, and the glucose so formed estimated by the method of Hagedorn & Jensen (1923). For this a Rehberg microburette was used; Krogh pipettes were used for the more critical measurements.

The histochemical work was done in the light of the recent work of Baker (1946) and Cain (1947) for fats, and Smyth & Hopkins (1948) for glycogen. While living coelomic cells in all forms were examined whenever possible, permanent preparations for fat distribution were made of tissue fixed in Baker's formal-calcium, and for glycogen in Best's picro-formal fixative at 0° C. For the study of glycogen, tissues were kept in absolute alcohol at 0° C until embedded in ester wax as recommended by Smyth & Hopkins (1948).

Best's carmine method was used for subsequent staining against diastase controls as recommended by Pearse (1953). In the study of fats, frozen sections were cut of whole tissues fixed in formal-calcium. Smears were made of the living coelomic cells (without addition of albumin) and fixed in the same way. Sudan black B, acetylated according to the technique of Casselman (1954) was used to localize the fats, the stain being dissolved either in 70% alcohol, or in propylene glycol (Chiffelle & Putt, 1951), against hot pyridine controls. The latter method seemed to give more delicate results. Baker's acid haematin method (1946) for phospholipids, and Cain's nile blue method (1947), staining and differentiating at 37° C, were also used, Gurr's glycerine jelly being employed as mountant in most cases.

RESULTS

Amphitrite johnstoni

Fats

Determinations of total fats in various tissues at different seasons are given in Table 1, and the arithmetic means are compared graphically in Fig. 1. The values for all tissues are highest in August, and the difference between the August values and those for November are significantly different for all tissues. The differences in the other values for body wall and gut are not statistically significant, and the higher April value compared with the February value for the coelomic cells only possibly significant. It can be concluded from these figures that the gut wall has the highest concentration of fat in the body at all times, and that the value is highest in August and lowest in November shortly after spawning. This suggests that the gut is to a certain extent a fat store. The body has the lowest value for fat concentration, but this may be due to the large proportion of muscular tissue which has a lower fat content than the epidermis. It is possible that the fat content of the epidermal layers is very similar to that of the gut, and it is this layer which may cause the seasonal change in values for body wall. The variation in value for coelomic contents reflects the quantity of gametes present; the value is highest in August owing to the large quantity of mature gametes, and low in November after spawning. The fat content of the nearly ripe oocytes and the trephocytes is similar. In August-September the cells and the oocytes may be separated almost completely by centrifugation. Spermatids and sperm contain much less fat, but the total fat in the coelom of a ripe male is much the same as that of a ripe female. The results of determinations of twelve randomly selected worms is shown in Table 2. The mean wet weight of the females was 9·35 g, of which 2·86 g was represented by the coelomic fluid and 0·95 g by the coelomic cells. In a ripe female, therefore, about 36 g of fat is represented by the oocytes and trephocytes, which is about half the total quantity in the remainder of the body at this time. During the early stages of maturation of the oocytes (January–February) when they are similar in size to the trephocytes, sudan black B

TABLE 1. CONCENTRATION OF FAT IN *AMPHITRITE JOHNSTONI* AT DIFFERENT SEASONS (MG/G WET WEIGHT)

	August (1955)	November (1955)	January–February (1956)	April (1956)
Body wall	7·37	10·60	3·83	5·00
	16·60	10·30	7·00	8·90
	11·45	8·05	7·95	10·00
	15·00	3·65	6·25	15·90
	16·00	7·20	7·80	6·30
	15·20	—	8·10	5·90
Mean	13·60	7·96	6·80	8·70
Gut	38·40	31·80	42·50	30·50
	42·50	23·20	26·80	39·40
	55·20	14·90	35·10	44·50
	47·40	41·70	49·40	51·50
	54·70	19·90	30·10	47·20
	40·30	25·70	25·30	—
Mean	46·40	26·20	34·90	42·60
Coelomic cells	45·20	15·70	12·10	21·30
	35·90	12·00	12·40	21·10
	29·50	6·80	8·50	14·40
	52·80	1·20	9·30	7·60
	51·20	—	10·20	26·90
	36·50	—	11·60	—
Mean	41·85	8·90	10·68	18·30

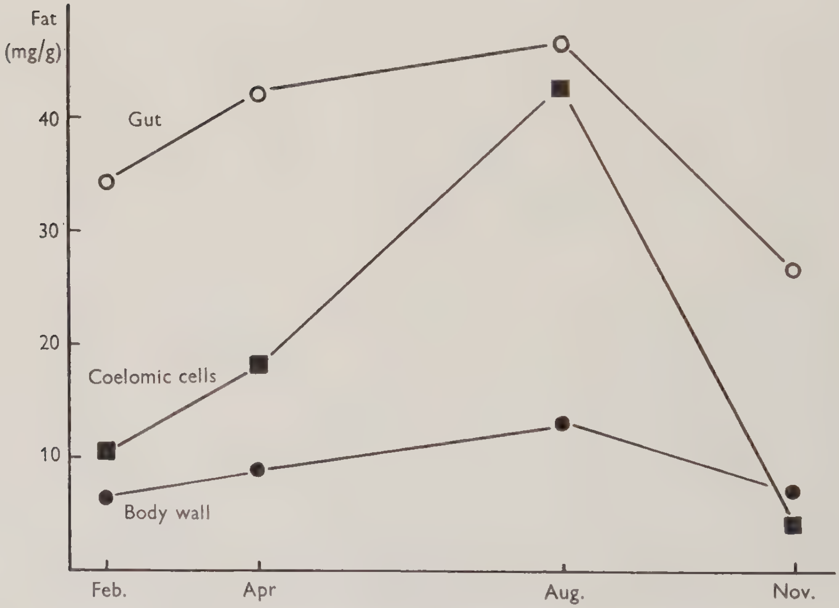


Fig. 1. Mean concentration of fat in *Amphitrite johnstoni* at different seasons (mg/g fresh weight)

staining showed no more fat in the oocytes than in the trephocytes. At this time, six worms of mean weight 9·0 g gave a mean total value of 6·1 mg of fat represented by the coelomic cells. There is about six times this quantity in the gut. The proportion by weight of the different parts of the body of three randomly selected worms is shown in Table 3.

TABLE 2. *AMPHITRITE JOHNSTONI*, CONCENTRATION OF FAT IN COELOMIC CONTENTS (MG/G WET WEIGHT)

	Trephocytes	Oocytes	Spermatids/sperm
Weight analysed	(1) 2·985 g (female)	(1) 4·927 g	—
	(2) 3·667 g (male)	(2) 7·332 g	(2) 334 g
Total fat	(1) 45·2	(1) 31·8	10·2
	(2) 35·9	(2) 42·2	—
Phospholipid	(1) 12·0	(1) 15·0	6·9
	(2) 15·3	(2) 19·4	—
Total sterols	(1) 3·3	(1) 3·5	1·8
	(2) 3·4	(2) 4·1	—

TABLE 3. *AMPHITRITE JOHNSTONI*, PROPORTION OF PARTS ANALYSED (G WET WEIGHT)

	Gross weight	Body wall	Gut	Coelomic cells
	10·6	3·6	0·76	1·25
	9·6	3·0	0·56	1·20
	9·1	2·4	0·70	1·20
Mean	9·8	3·0	0·67	1·22

TABLE 4. *AMPHITRITE JOHNSTONI*, CONCENTRATION OF FAT IN DIFFERENT PARTS OF THE GUT (MG/G)

Fore stomach	Hind stomach	Intestine
61·8	14·0	82·5
87·3	18·0	56·2

The concentration of fat in different parts of the gut was investigated, and the results of two determinations each containing guts from six worms, are shown in Table 4. The values for the fore stomach and intestine are not significantly different, but the hind stomach, which is almost entirely muscular (Dales, 1955), is much lower and this value is interesting in giving some idea of the fat content of muscle alone. The values for fore stomach and intestine are higher than for the trephocytes, though the greater weight of the trephocytes tends to equate the total amount of fat represented in the gut and the trephocytes in any worm.

Glycogen

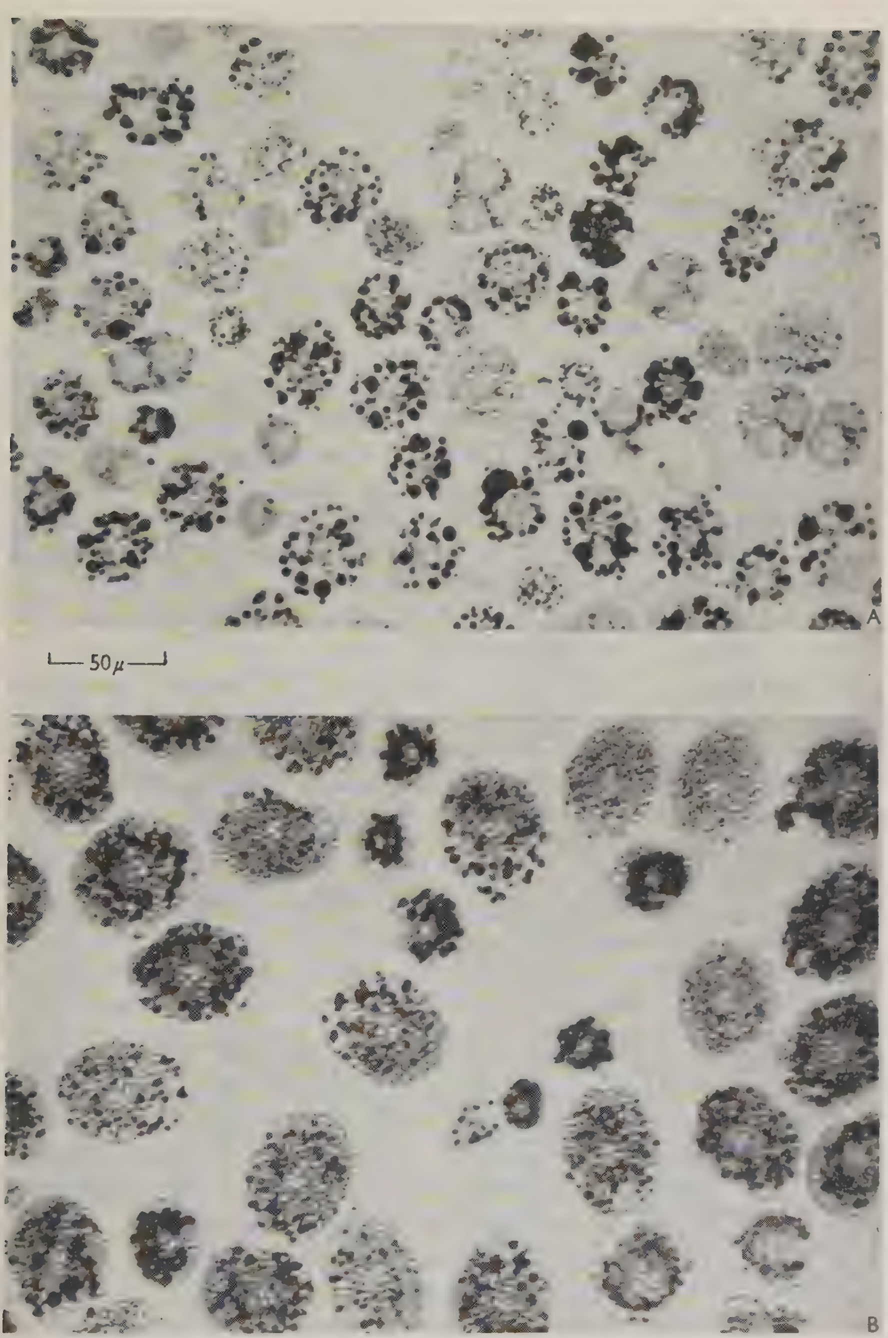
The concentration of glycogen in different parts of the body is shown in Table 5. The low concentration in all parts of the gut contrasts strongly with the high fat values, though the concentrations in the body wall and the coelomic cells are roughly equivalent to the concentration of fats. Sections of body wall stained with Best's carmine showed most of the glycogen in the peritoneum, and as there is much less in the muscular layers which constitute a large proportion of the weight, the actual concentration in the peritoneum may be high. It is interesting that the values for the coelomic cells are not very different from the values for fat.

TABLE 5. *AMPHITRITE JOHNSTONI*, CONCENTRATION OF GLYCOGEN IN DIFFERENT PARTS OF THE BODY (MG/G WET WEIGHT)

			Gut		
	Body wall	Coelomic cells	Fore stomach	Hind stomach	Intestine
	13·7	37·4	3·5	2·3	6·2
	11·6	18·1	5·8	1·4	2·0
	6·9	35·0	3·6	1·7	2·3
	10·5	13·3	3·6	1·4	3·5
	19·2	29·2	7·8	3·3	2·8
	14·2	36·0	5·8	2·0	10·4
	5·3	15·5	5·3	2·2	1·5
	10·5	44·9	2·8	2·3	3·7
	14·7	18·9	6·3	1·8	2·8
	9·7	26·4	6·7	1·9	5·5
	13·1	16·8	—	1·0	3·5
	17·4	8·4	—	—	2·7
	—	16·8	—	—	—
	—	32·0	—	—	—
Mean	12·2	25·7	5·1	1·8	3·6

Histochemical data

Smears of the coelomic cells were made at all seasons and stained for fats and glycogen. Their appearance when stained with sudan black B is shown in Fig. 2. All the granules stain a dark black and the same granules stain dark blue with Cain's 0·02% nile blue and give a positive reaction with Baker's acid haematin, so that they are at least partly phospholipid in nature. No red-staining granules were ever found, either in the trephocytes or in other parts of the body with Cain's method, and positive results were always obtained with Baker's acid haematin test. From the quantitative data it is known that 30–50% of the total fatty acids in most tissues is phospholipid, so that these granules are presumably a mixture of neutral fat and phospholipid or possibly consist of neutral fat surrounded by a phospholipid shell. Purple-staining granules were in fact sometimes obtained with Cain's method, and the smaller granules of fat nearer the nucleus in the trephocytes often gave a rather greenish colour with 0·02% nile blue. These granules are about 2 μ in diameter, the largest about 10 μ in diameter. Glycogen is scattered throughout the cytoplasm and these granules are only 1–2 μ in diameter. Frozen sections of gut and body wall were made and stained with acetylated sudan black B. In the gut most of the fat is present in the epithelial cells rather than in the very thin peritoneum, and in the body wall most of the fat is in the epidermal layer.



ig. 2. Coelomic trephocytes of *Amphitrite johnstoni*, stained with acetylated sudan black B in ropylene glycol. A: cells from the dark form showing the large unstained spherical granules. : cells from the light form, lacking the spherical granules.

Dark and light forms

Adult *Amphitrite johnstoni* vary in external appearance from a light pink to a dark brown colour. The trephocytes have the same colour in the mass as the epidermis, and the dark worms have many trephocytes containing large granules (Fig. 2A) which are absent from the pink form. The granules are roughly spherical and may be 12–15 μ in diameter; they are unstained by fat stains or Best's carmine. In the work on the heart body (Kennedy & Dales, unpublished) extracts were made of the dark and light trephocytes and examined by long paper chromatography (Kennedy, 1953). While these results will be published elsewhere, it may be noted here that the dark trephocytes were found to contain coproporphyrin III, which was absent in the light trephocytes. Both dark and light cells contained a haematin. A haematin was found also in the trephocytes of *Terebella* which, unlike those of *Amphitrite*, contain haemoglobin. The dark non-fluorescent spots obtained in the long paper chromatography became fluorescent after treatment with hydrazine hydrate and gave an R_F value of 0·81 in *Terebella* and both types of cell in *Amphitrite*, indicating that the original dark spot was a haematin of a dicarboxylic porphyrin, probably protoporphyrin.

Terebella lapidaria

A few determinations were made on the fats in this species during the search for an animal which might show the effect of starvation on the fats in the different tissues. *Amphitrite* does not starve well in the laboratory; *Terebella* is rather more hardy but the experiment was continued for a short time only (10 days), and the results show no effect of starvation on the fats.

TABLE 6. *TEREBELLA LAPIDARIA*, CONCENTRATION OF FAT IN VARIOUS TISSUES (MG/G WET WEIGHT)

	Body wall	Gut	Coelomic cells
Controls	26·3	35·7	32·8
'Starved'	20·0	32·9	32·5

Worms were kept in cleaned sand under glass plates, the circulating water being passed through bolting silk. Determinations of the fat in the body wall, gut and trephocytes in twelve control and twelve of the starved animals showed no difference in the fat content (Table 6). Nevertheless, it is interesting that the trephocytes have a concentration of fat similar to that in *Amphitrite*, though they differ in having haemoglobin as well.

Arenicola marina

Fats

The quantity of coleomic cells is very small at all seasons, though there is some increase during the maturation of the gametes, though even then the quantity of cells in the coelomic fluid is small compared with the quantity of gametes. The quantity of coelomic cells was measured at different times by filtering the whole of the coelomic fluid through oven-dried and weighed filter-paper and reweighing after drying at 100° C. It was found that the value did not differ significantly over most of the year, and that the increase in weight of coelomic contents towards October was due entirely to the oocytes, which may be separated from the other cells by centrifugation. The detailed results are of little relevance in the present context, but it may be noted here that of sixteen worms of mean weight 3·15 g sampled in January, the mean dry weight of the coelomic cells was 5·6 mg, or less than 0·2 % of the gross weight of the worm. While the quantity of coelomic cells is much less than in *Amphitrite*, the concentration of fat is not so much less (Table 7). *Arenicola* starves better in the laboratory than most worms, and in November–December worms were kept for 6 weeks at room temperature without food. Those that survived had lost considerable weight, though much of this reduction was due to loss of coelomic fluid. Determinations were made on the fats of normal and starved worms, and it was found that in starved worms there was a greatly increased fat-concentration in the gut and body wall, but the coelomic cells did not seem to be affected (Table 7). In a normal non-breeding worm with a gross weight of 8·0 g, a total of 47·5 mg of fat is located in the body wall, 18·1 mg in the gut and less than 0·2 mg in the coelomic cells.

TABLE 7. *ARENICOLA MARINA*, CONCENTRATION OF FAT IN VARIOUS TISSUES (MG/G WET WEIGHT)

(November–December: six worms in each determination.)

		Body wall	Gut	Coelomic cells
Normal		13·95	33·20	15·80
		11·20	39·70	26·00
		11·95	13·10	39·00
	Mean	12·33	28·67	26·93
Starved (6 weeks)		21·75	123·50	23·60

Glycogen

The results of determinations of the quantity of glycogen present in various tissues in six randomly selected worms is shown in Table 8. The values for the coelomic cells were negligible and have not been included. It will be seen that the concentration of glycogen is highest in the body wall, the value being of the same order as that in the trephocytes of *Amphitrite*.

TABLE 8. *ARENICOLA MARINA*, CONCENTRATION OF GLYCOGEN IN VARIOUS TISSUES (MG/G WET WEIGHT), AND PROPORTION OF PARTS (APRIL)

	Gross weight (g)	Weight coelomic contents (g)	Weight body wall (g)	Concentration glycogen in body wall (mg/g)	Weight gut (g)	Concentration glycogen in gut (mg/g)
	3·2	1·6	1·3	25·1	0·3	11·4
	9·7	4·0	5·3	22·5	0·6	10·6
	6·0	3·1	2·6	20·0	0·3	6·3
	11·0	4·7	5·3	20·7	1·0	15·6
	11·8	4·5	6·2	12·1	1·1	11·7
	5·3	2·5	2·4	39·4	0·4	13·9
Mean	—	—	3·8	23·3	0·6	11·6

Histochemical data

Sudan black B staining showed that fat granules were present in some of the ceolomic cells, but never in great quantities. The oocytes, too, contain little fat as compared with either *Amphitrite* or *Nereis*. Glycogen was not demonstrable by Best's carmine in the coelomic cells. In sections of body wall considerable deposits were found in the peritoneum, while fat occurs mainly in the gut epithelium and the epidermis.

Nereis diversicolor

Fats

In a previous paper (Dales & Kennedy, 1954) the seasonal variation in colour in this species was described and it was suggested that the greener specimens most usually encountered in early spring owed their colour to the withdrawal of carotenoids from the epidermis thereby unmasking the biliverdin to which the green colour is due. Determinations of the fat content of the body wall in different worms have been made before and after spawning in order to test the hypothesis that the carotenoid removal is merely a visible manifestation of the withdrawal of the epidermal fats in connexion with the maturation of the oocytes. The results are not conclusive, but are presented here because of the interest of the values for fat and glycogen in the general economy of the animal.

Completely green males just before spawning have an extremely thin body wall and this has a fat content below that measurable in a single worm by the method used. While fats have been removed along with other substances in the tissues phagocytized, there is no evidence that they are withdrawn specifically for utilization in the maturation of the sperm. In ripe females, which are usually greener soon after the spawning period, the fat content is still high. The values for November, January (pre-spawning), and April (post-spawning) do not differ significantly (Table 9). There may still be a real utilization of epidermal fat in the maturation of the oocytes, but the high proportion of muscle included in the body-wall samples analysed may mask the change, for there is no doubt that a green male has negligible amounts of fat in the epidermis while a non-breeding worm with an orange hue has values in the order of 16 mg/g. The amount of fat represented by the sperm in the coelom is roughly equivalent to that represented by the oocytes in a mature female of similar size. In April it was found that the gut contains about 20 mg/g of fat.

TABLE 9. *NEREIS DIVERSICOLOR*, CONCENTRATION OF FAT IN THE BODY WALL AT DIFFERENT TIMES (MG/G WET WEIGHT). FEMALES

	November (1955)	January (1956)	April (1956)
	17·00	18·40	10·50
	15·70	15·40	13·50
	17·90	7·96	15·90
	27·50	23·70	20·00
	27·10	22·40	11·50
	11·50	12·85	15·75
	13·70	20·10	13·10
	12·00	20·90	—
	21·00	—	—
	9·80	—	—
Mean	17·32	17·70	14·32

TABLE 10. *NEREIS DIVERSICOLOR*, ANALYSIS OF OOCYTES

	Fat concentration (mg/g)	Glycogen concentration (mg/g)	Water (% wet weight)
16 Jan. (coelomic)	59·1	32·9	20·9
19 Feb. (released)	97·0	57·5	57·5

TABLE 11. *NEREIS DIVERSICOLOR*, TOTAL WEIGHT OF OOCYTES IN RIPE FEMALES (g)

	Gross weight of worm	Wet weight oocytes
	0·489	0·0423
	0·790	0·0498
	0·602	0·0577
	0·621	0·0550
Mean	0·625	0·0510

In a ripe female of 625 mg gross weight, the coelomic fluid weighs about 75 mg. The tissues have a fat content of about 15·7 mg/g and the ripe eggs 90 mg/g (Table 9). A worm of this size has about 50 mg of eggs, or a total of 4·5 mg of fat in the eggs—about half the quantity in the body wall (Table 9). If all the fat required for the maturation of the oocytes were withdrawn from the body wall alone, a much greater variation in the body wall values would be expected. The fat and glycogen content of the oocytes are shown in Table 10. Clearly, either only some of the fat is withdrawn from the body wall or there is a continual replacement of fat, implying a resorption of tissue from other sites or continued feeding by the animal prior to spawning. It has been remarked already (Dales & Kennedy, 1954) that females remain much more healthy prior to spawning than the males, in which much more phagocytosis occurs and which may well be incapable of feeding just before the spawning period. As far as the males are concerned all the fat required for the maturation and nourishment of the sperm could easily be provided by the amount in the body wall alone.

Glycogen

Some determinations of the concentration of glycogen in the body wall were made in January. The mean value of ten determinations was 34·3 mg/g, a value which is high compared with *Arenicola* or *Amphitrite*.

TABLE 12. FAT CONCENTRATION IN DIFFERENT TISSUES (MG/G)

	Body wall	Gut	Coelomic cells (excluding oocytes)
Amphitrite	9·26	37·50	40·50
Arenicola	12·33	28·67	26·93
Terebella	26·30	35·70	32·80
Nereis	15·70	21·40	—

DISCUSSION

In Table 12 are summarized all the mean values of fat concentration in the four species studied. It will be noticed that there is a rough correspondence in each species between the values for gut and coelomic cells. While *Arenicola* and *Amphitrite* are similar in this respect, the proportion which each part represents in the total body weight is different. If the total quantities of fat are calculated for each species in worms of similar size (say 10 g) it appears that the total quantity of fat in each is similar, and the lack of a well-developed trephocyte system in *Arenicola* is compensated for by a larger proportion of body wall in the total body weight. This is shown in Table 13. The same comparison may be made for the total quantity of glycogen present, and these calculations are shown in Table 14.

It is difficult to conclude from these preliminary results how far these variations reflect different food depots, but the increase in fat concentration in all tissues in late summer in *Amphitrite* and a corresponding decrease in winter suggests that the fats are both deposited and withdrawn from the gut, the body wall, and the trephocytes during the maturation of the gametes. The high fat values for the gut and the histochemical evidence of its distribution in the epithelial cells suggests that the physiological difficulty of transporting fats in the blood is partly overcome by storing them primarily in the epithelial cells of the gut itself, though perhaps later the fat may be transferred by the blood stream to the epidermis, by the peritoneum to the coelomic fluid, or may be removed by amoebocytes. Except in the event of direct removal by amoebocytes, the fat must first enter the blood stream, since the epithelial cells are separated from the muscular and peritoneal layers by the gut sinus. This interpretation would concur with the views of Romieu (1923) and Kollman (1908) that the trephocytes are amoebocytes which have taken up fat granules.

The similar appearance of the epidermis and coelomic cells that has been noted in *Nereis* (Dales & Kennedy, 1954), *Owenia* (Dales 1957) and in the dark and light forms of *Amphitrite* (Kennedy & Dales, unpublished), suggests a close interrelationship between them. Fretter (1953) found that the amoebocytes in *Platynereis dumerilii* took up radioactive strontium and yttrium from the sea water, both through the gut and the epidermis, and that amoebocytes containing these isotopes were most frequent in the epidermis. Fretter, following Liebman (1946), distinguishes these amoebocytes from trephocytes which did not, apparently take up the isotopes. In *Amphitrite* trephocytes in the coelom certainly show no amoebic powers, though they may be kept healthy in a hanging drop in a sealed slide for many days. Fauré-Fremiet's observations (1929) confirm this. The amount of fat increases with increasing size of the trephocyte, but the largest contain few granules. This suggests that the fat is either synthesized by the cell or taken up directly from the coelomic fluid, and eventually discharged for the benefit of younger cells or growing gametes. It is just possible that there is some correspondence between the glucose in the body fluid, and the glycogen and fat in the trephocyte, and it is planned to investigate this further. Seton & Wilbur (1949) have made some determinations of the glucose in the coelomic fluid, which was found to increase with rising temperature. In some preliminary determinations in *Amphitrite* in the present work, the glucose content was found to increase under various adverse conditions at different temperatures, but insufficient observations have been made as yet to make any conclusions about these experiments.

TABLE 13. TOTAL QUANTITY OF FAT IN 10 G WORMS (MG) (NOVEMBER–FEBRUARY)

	Body wall	Gut	Coelomic cells	Total
Arenicola	55·3	20·0	0·135	75·4
Amphitrite	27·8	25·1	17·400	70·3

TABLE 14. TOTAL QUANTITY OF GLYCOGEN IN 10 G WORMS (MG) (APRIL)

	Body wall	Gut	Coelomic cells	Total
Arenicola	88·5	7·0	—	95·5
Amphitrite	37·0	1·9	36·6	74·5

The trephocytes in *Amphitrite* undoubtedly contain substances other than glycogen and fat, but these have not yet been determined and are beyond the scope of this paper. Both Fauré-Fremiet (1929) and Romieu (1923) state that albuminoids are present, and Romieu (1923) also identifies various excretory substances. Von Brand (1927), Fauré-Fremiet (1929) and Wilbur & Bayors (1947) are the only previous workers who have published determinations of fat content in polychaete tissues. Fauré-Fremiet (1929) found a total fat content in the trephocytes of *Amphitrite* of 1·23% (wet weight), and Wilbur & Bayors (1947) found a total lipid content in *Amphitrite ornata* of 3·15%, *Arenicola marina* 1·22% and *Nereis pelagica* of 2·17% of the total wet weight. Von Brand (1927) found that fat accounted for 3·81% in *N. pelagica*, 0·54 in *A. marina* (wet weight). These values are not dissimilar to those obtained in the present work, but they have little relevance to the present problem since they are overall values, and no information is given about the state of the worms. Alscher (1949) has also made observations on the trephocytes in *Arenicola marina* and *Amphitrite ornata* and has made counts of the number of fat granules in different types and size of coelomic cell. The counts show that the amount of fat present within each size-group of cell is very variable, but in both species the smaller cells have fewer granules, and the largest cells have the widest spread in number with a possible inference of loss of granules in the larger and older cells.

While the experiments on the effect of starvation are not conclusive, the rise of fat concentration after starving in *Arenicola* is very reminiscent of the 'fatty liver' condition in vertebrates, but it may be due directly to substances other than fats being withdrawn at times of inanition. On the other hand, in the male *Nereis diversicolor* the gut and the body wall are eroded almost completely immediately before the spawning period, and the total fat content is considerably less than its normal value. It is proposed to examine the effects of starvation more closely, especially in relation to glycogen which has not yet been measured in starving worms.

The increase in the number of trephocytes during the maturation of the gametes in *Nereis* (Dales, 1950) suggests that these cells are primarily, if not solely, concerned with this function. The terebellids, on the other hand, maintain a large number of trephocytes which does not vary appreciably during the year, though there is a considerable loss when spawning occurs. While Liebman (1946) may be correct in assuming a dual origin for the amoebocytes and trephocytes in earthworms, there is no evidence for this in *Amphitrite*. The assumption that the trephocytes in earthworms are chloragocytes which have become free may be correct, but these trephocytes may not be comparable with the trephocytes in *Amphitrite*. Terebellids do not appear to have any cells on the outside of the gut or on the dorsal vessel which could be described as chloragocytes, and green or yellow granules are found only in the heart body (Kennedy & Dales, unpublished). There is no evidence that the trephocytes in *Amphitrite* are derived from the heart body, or that this organ ever liberates cells into the blood stream in which they are virtually absent. Ashworth (1904) describes intravasal tissue in the heart of *Arenicola*, but this is very small compared with that in *Amphitrite*. Kermack (1955) has given some account of the activity of amoebocytes after injection of ink particles into the gut of *Arenicola*, amoebocytes laden with ink particles collecting in the intravasal tissue and in the coelom. This is interesting in demonstrating one way in which substances may be removed directly from the gut. The intravasal tissue contains chloragosomes, but chloragocytes are found also in the coelomic epithelium (Schneider, 1899). The relationships of the chloragocytes and the amoebocytes and trephocytes are not known. Some of these problems are now being investigated.

SUMMARY

Determinations of the concentration of fat and glycogen in the body wall, in different parts of the gut and in the coelomic cells are described in *Amphitrite* and *Arenicola*. It is suggested that the trephocyte system constitutes a store of fat and glycogen derived from a primary store in the absorptive parts of the gut itself. In *Arenicola* and *Nereis* surplus fat is removed from the gut itself through the blood or directly by amoebocytes; the fat deposited in the epidermis and the glycogen in the peritoneum. In these worms the coelomic trephocytes are solely concerned with the maturation of the gametes. No relationship can be established between these cells and the chloragocytes. In *Amphitrite* and *Terebella* fat is stored also in the coelomic trephocytes which may derive their contents directly from the gut or from the body wall. Glycogen is stored in the trephocytes in *Amphitrite*, and in *Arenicola* in the peritoneum. Thus while large amounts of fat and glycogen are found in the trephocytes in *Amphitrite*, the total amount present in the body is no more than in *Arenicola* which lacks a well-developed trephocyte system, and in this species a larger proportion of fat and glycogen is found in the body wall.

REFERENCES

ABDEL-FATTAH, R. F., 1955. The chloragogen tissue of earthworms and its relation to urea metabolism. *Proc. Egypt. Acad. Sci.*, Vol. 10, pp. 36–50.

ALSCHER, R. P., 1949. Description of, and localization in, cells of body cavity fluid of *Arenicola marina* (Lamarck) and *Amphitrite ornata* (Leidy). *Biol. Bull., Woods Hole*, Vol. 97, pp. 253–4.

ASHWORTH, J. H., 1904. Arenicola. *L.M.B.C. Memoirs*, Vol. 11, 118 pp. London: Williams and Norgate.

BAKER, J. R., 1946. The histochemical recognition of lipine. *Quart. J. micr. Sci.*, Vol. 87, pp. 441–70.

BLOOR, W. R., 1928. The determination of small amounts of lipid in blood plasma. *J. biol. Chem.*, Vol. 77, pp. 53–73.

—— 1929. The oxidative determination of phospholipid (lecithin and cephalin) in blood and tissues. *J. biol. Chem.* Vol. 82, pp. 273–86.

BRAND, T. F. VON, 1927. Stoffbestand und Ernährung einiger Polychaëten und anderer mariner Würmer. *Z. vergl. Physiol.*, Bd. 5, pp 643–98.

CAIN, A. J., 1947. The use of nile blue in the examination of lipoids. *Quart. J. micr. Sci.*, Vol. 88, pp. 383–92.

CASSELMAN, W. G. B., 1954. Acetylated sudan black B as a more specific histochemical reagent for lipides. *Quart. J. micr. Sci.*, Vol. 95, pp. 321–2.

CHIFFELLE, T. L. & PUTT, F. A., 1951. Propylene and ethylene glycol as solvents for sudan IV and sudan black B. *Stain Tech.*, Vol. 26, pp. 51–6.

CLAPARÈDE, E., 1868. *Les annélides chétopodes du Golfe de Naples.* 500 pp. Geneva: Georg.

—— 1873. *Recherches sur la structure des Annélides Sédentaires.* 200 pp. Geneva: Georg.

CUÉNOT, L., 1891. Études sur le sang et le glandes lymphatiques dans la série animale. *Arch. Zool. exp. gén.*, Sér. 9, T. 2, pp. 365–475.

—— 1898. Études physiologiques sur les Oligochètes. *Arch. biol.*, T. 15, pp. 79–124.

DALES, R. P., 1950. The reproduction and larval development of *Nereis diversicolor* O. F. Müller. *J. mar biol. Ass. U.K.*, Vol. 29, pp. 321–60.

—— 1955. Feeding and digestion in terebellid polychaetes. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 55–79.

—— 1957. The feeding mechanism and morphology of the gut of *Owenia fusiformis* delle Chiaje. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 81–9.

DALES, R. P. & KENNEDY, G. Y., 1954. On the diverse colours of *Nereis diversicolor. J. mar. biol. Ass. U.K.*, Vol. 33. pp. 699–708.

FAURÉ-FREMIET, E., 1929. Constitution et proprietes physico-chimiques des éléocytes d'*Amphitrite johnstoni* (Malmgren). *Protoplasma*, Vol. 5, pp. 321–37.

FRETTER, V., 1953. Experiments with radioactive strontium (^{90}Sr) on certain molluscs and polychaetes. *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 367–84.

FREUDWEILER, H., 1905. Studien über das Gefäßsystem niederer Oligochäten. *Jena. Z. Naturw.*, Bd. 40, pp. 383–422.

HAGEDORN, H. C. & JENSEN, N., 1923. Zur Mikrobestimmung des Blutzuckers mittels Ferricyanid. *Biochem. Z.*, Bd. 135, pp. 46–58.

HERPIN, R., 1921. Sur l'origine et le rôle des cellules à résèrves de la cavité générale chez *Perinereis cultrifera* (Gr.) et *Perinereis Marioni* (Aud. et Edw.) et la différenciation précoce de leurs œufs. *C.R. Acad. Sci., Paris.*, T. 173, pp. 249–52.

ISSEL, R., 1905. Intorno agli escreti dei linfociti. *Arch. Zool.* (*ital.*), *Napoli*, Vol. 2, pp. 125–35.

KENNEDY, G. Y., 1953. Partition paper chromatography of the porphyrins. *Scand. J. clin. Lab. Invest.*, Vol. 5, pp. 281–4.

KERMACK, D. M., 1955. The anatomy and physiology of the gut of the polychaete *Arenicola marina* (L.). *Proc. zool. Soc. Lond.*, Vol. 125, pp. 347–81.

KOLLMAN, M., 1908. Notes sur les réserves albuminoides des Insectes et des Annélides. *Bull Soc. zool. Fr.*, T. 34, pp. 149–55.

LIEBMAN, E., 1946. On trephocytes and trephocytosis; a study on the role of leucocytes in nutrition and growth. *Growth*, Vol. 10, pp. 291–330.

PEARSE, A. G. E., 1953. *Histochemistry, Theoretical and Applied*, 530 pp. London: Churchill.

PICTON, L. J., 1898. On the heart body and coelomic fluid of certain polychaetes. *Quart. J. micr. Sci.*, Vol. 41, pp. 263–302.

RICE, W. J., 1902. Studies in earthworm chloragogue. *Biol. Bull., Woods Hole*, Vol. 3, pp. 88–94.

ROMIEU, M., 1921. Les inclusions cristallines des éléocytes de *Nereis* et leurs relations avec la granulation éosinophile. *C.R. Acad. Sci., Paris*, T. 168, pp. 367–9.

—— 1923. Recherches histophysiologiques sur le sang et sur le corps cardiaque des Annélides polychètes. *Thès. Fac. Sci. Univ. Paris*, 336 pp., Paris: Doin.

ROSA, D., 1896. I linfociti degli Oligocheti. Ricerche istologiche. *Mem. R. Accad. Torino*, Vol. 46, pp. 149–78.

ROSA, D., 1898. I pretesi rapporti genetici tra i linfociti ed il chloragogeno. *Atti Accad., Torino*, Vol. 33, pp. 612–37.

SCHAEPPI, T., 1894. Das Chloragogen von Ophelia radiata. *Jena Z. Naturw.*, Bd. 28, pp. 249–93.

SCHNEIDER, G., 1896. Über phagocytäre Organe und Chloragogenzellen der Oligochäten. *Z. wiss. Zool.*, Bd. 61, pp. 363–92.

—— 1897. Über die Segmentalorgane und den Herzkörper einiger Polychäten. *Arb. d. Kaiserl. Naturf. Ges. St. Petersburg*, Vol. 27, pp. 135–7.

—— 1899. Über Phagocytose und Excretion bei den Anneliden. *Z. wiss. Zool.*, Bd. 66, pp. 497–520.

SETON, E. & WILBUR, G. G., 1949. Glucose metabolism in marine annelids. *Biol. Bull., Woods Hole*, Vol. 97, p. 246.

SMYTH, J. D. & HOPKINS, C. A., 1948. Ester wax as a medium for embedding tissue for the histological demonstration of glycogen. *Quart. J. micr. Sci.*, Vol. 89, pp. 431–5.

SPERRY, W. M., 1938. A micromethod for the determination of the total and free cholesterol. *Amer. J. clin. Path., Tech. Suppl.*, Vol. 2, pp. 91–9.

STERLING, S., 1908. Das Blutgefäßsystem der Oligochäten. *Jena Z. Naturw.*, Bd. 44, pp. 253–352.

WILBUR, C. G. & BAYORS, W. M., 1947. A comparative study of the lipids in some marine annelids. *Biol. Bull., Woods Hole*, Vol. 93, pp. 99–101.

WILLEM, V. & MINNE, A., 1900. Recherches sur l'excrétion chez quelques Annélides. *Mém. Acad. R. Belg. Cl. Sci.*, T. 58, pp. 1–73.

APPENDIX

ANALYSIS OF FATS

Extraction

Tissues were dried on filter-paper and the quantity extracted weighed by difference before being macerated in a Griffith pattern tissue grinder. Extraction was effected by refluxing with fifty times the weight of the tissue with 95% ethanol:peroxide-free ether 3:1 mixture overnight. Larger quantities of tissue were extracted in a Soxhlet apparatus. The extract was filtered through fat-free paper and made up to 50 ml. The efficiency of extraction was tested with known quantities (50–100 mg) of pure oleic acid absorbed on filter paper.

Estimation of total fatty acids plus sterols

A 20 ml. portion of the extract was measured accurately into a wide-necked Ehrlenmeyer flask, and 2 ml. of sodium ethoxide added. This was then placed in a ventilated electric oven at 90° C and allowed to evaporate to about 2 ml. This was found more satisfactory than evaporating on a water-bath owing to loss by spitting. The pasty residue was dried in a current of air, and the fatty acids liberated by the addition of 1 ml. of dil. H_2SO_4 and heating on a boiling water-bath for 1 min. The fatty acids were then dissolved in successive 3 ml. portions of boiling 40–60 light petroleum by rotating over the water-bath for 3 min, the combined extracts being made up to 25 ml. A 10 ml. portion of this was carefully measured into a 150 ml. conical ground glass-stoppered flask and evaporated to dryness, the last traces being blown off in a current of air. A control flask was started at this stage. 5 ml. of the silver chromate: H_2SO_4 reagent and exactly 3 ml. of 1·0 N potassium dichromate were then added, the contents mixed, the flask stoppered and placed in the oven at 90° C for 90 min. In the event of all the oxidant becoming used up after 5 min, the light petroleum extract was diluted, and the process repeated. The flasks were placed on a bed of sand in the oven to maintain a more even temperature. After 90 min the contents were diluted to about 100 ml. with cold distilled water and carefully washed out into a 250 ml. conical flask; 10 ml. of 10% potassium iodide added and the mixture immediately titrated against 0·1 N sodium thiosulphate, using starch indicator. The difference in the titration values between the samples and the control is a measure of the amount of oxidant reduced by the fatty acids. Usually four flasks were run at once. Values within ±2% were obtained with pure oleic acid. All steps in the procedure were tested, and most loss occurs in the oxidation.

Estimation of phospholipids

A 20 ml. portion of the ethanol:ether extract was evaporated to dryness in a 50 ml. beaker. The residue was then extracted with 3 ml. portions of boiling 40:60 light petroleum and the extracts combined in a 15 ml. centrifuge tube. Any sediment was centrifuged out and the clear liquid decanted into another tube if necessary and allowed to evaporate to 2 ml. To the solution was then added 7 ml. of acetone and 0·1 ml. of magnesium chloride in ethanol (saturated solution), the solutions mixed and the phospholipid precipitate centrifuged down. The supernatant liquid was then carefully drawn off, the gummy precipitate kneaded with successive portions of acetone, and finally allowed to dry by inverting the tube on filter-paper. The precipitate was then dissolved in moist ether, with warming, and the solution transformed to the 150 ml. oxidation flask, the ether evaporated in a current of air and the phospholipid estimated by the chromic acid oxidation mixture as before. Bloor's correction factor was applied in the calculations.

Estimation of total sterols (free and combined)

Exactly 1 ml. of the ethanol:ether extract was transferred to a 15 ml. centrifuge tube, 0·05 ml. of 50% KOH added and the mixture warmed to 40° C for 30 min. After cooling the mixture was neutralized with 5% HCl using phenolphthalein as indicator. 1 ml. of 1% digitonin in 50% ethanol was added, stirred with a glass rod and allowed to stand 24 h. The precipitate was centrifuged down at 2500 r.p.m. for 15 min, the supernatant carefully removed, the rod replaced and the tube vigorously washed down with 2 ml. acetone:ether 1:2 mixture from a syringe; after further centrifugation, the precipitate was washed and centrifuged twice with pure ether, the precipitate being then dried at 40° C in a current of air. The tube was kept in a desiccator until ready to develop the colour reaction.

The precipitate was then dissolved in 1 ml. glacial acetic acid; warming to 40° C and stirring with original glass rod was usually necessary. Exactly 2·1 ml. of acetic anhydride:H_2SO_4 mixture was added and the tube with controls stirred well and placed in an incubator at 25° C for exactly 30 min. The density of the colour was measured at 670 mμ using a 'Unicam' S.P. 600 Quartz spectrophotometer, and the amount of sterol present calculated from a standardization curve obtained by successive dilution of a known solution of pure cholesterol.

The above description includes all the data on the variations in the general methods of Bloor (1928, 1929) and Sperry (1938). Reference should be made to the original papers for details of making and purity of the reagents required.

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A NOTE ON *STICHOCOCCUS BACILLARIS* NAEG. AND SOME SPECIES OF *CHLORELLA* AS MARINE ALGAE

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(Text-fig. 1)

The recent publication (Butcher, 1952) of some new marine algal species belonging to genera largely non-marine arouses interest as there are relatively few genera ranging from marine to freshwater habitats. Two of the genera concerned, *Chlorella* and *Stichococcus*, are notable for their very wide habitat range in fresh water and on land.

Three marine *Stichococcus* strains were examined and compared with *S. bacillaris* Naegeli, 1849, and with various non-marine strains of that species. The marine strains were (i) *Stichococcus cylindricus* Butcher, type strain; (ii) another strain identified by Butcher as *S. cylindricus*; and (iii) Plymouth no. 82 = Cambridge no. 379/5, also identified by Butcher as *S. cylindricus*. None of these three strains differed appreciably from Naegeli's description of *S. bacillaris*. The shape and dimensions agree, while the presence or absence of a pyrenoid cannot be regarded as decisive. Fritsch (1935) and Smith (1950) say that *Stichococcus* has no pyrenoid, while Prescott (1951) states there is one pyrenoid but does not depict it in his illustrations. Forest (1954) is probably nearer the truth when, referring to *Ulothrix* Kütz. em. Forest (including *Ulothrix*, *Hormidium* and *Stichococcus*), he writes: 'In only one species was the pyrenoid distinctive enough to be of possible critical value'. And that was not a species of *Stichococcus* Naeg. Though the tendency to fragment is strong in these strains the method of cell division appears typical of the genus and not intermediate between it and *Nannochloris* as Butcher suggests. The type strain when grown in a sea water and soil biphasic medium produces filaments of usually four to eight cells and occasionally up to 18 cells; while in the other strains seldom are more than two cells seen in a filament.

That Butcher's alga is both aquatic and marine does not warrant its position in a separate species. Though *S. bacillaris* is frequently found in damp terrestrial places it is often also aquatic and like *Chlorella* is one of the more usual colonists of neglected stock bottles of various solutions and of distilled water. It is not surprising therefore that this alga should be found in estuarine and off-shore marine waters.

To test the impression that *S. bacillaris* is an unexacting plant regarding salinity three strains were grown in (*a*) Föyn's erdschreiber medium, (*b*) the same but with half-strength sea water, and (*c*) a similar medium with no sea water added. The strains were, a freshwater strain, Cambridge 379/1 c; a strain isolated from the contents of a cow's rumen, Cambridge 379/1 e; and the marine Cambridge 379/5. They were grown in the different media for 10 days before being inoculated into the cultures for estimation. Haemacytometer counts were made covering the logarithmic phase of growth. Irregularity in some of the counts was due largely to the cells clumping or adhering to the glass despite vigorous shaking. The growth curves (Fig. 1 A) suggest that the marine form grows equally well in fresh or sea water. The freshwater form is equally tolerant but with lower maximum cell counts. Strain 379/1 e grew

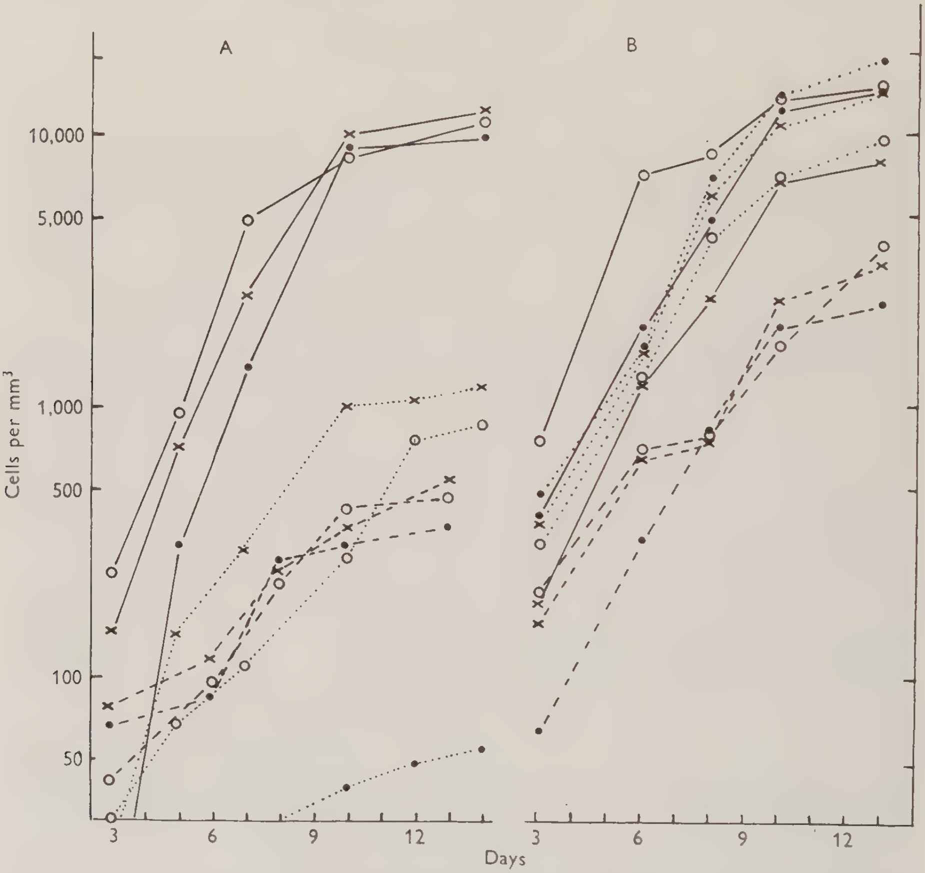

Fig. 1. Growth of (A) *Stichococcus* cultures and (B) *Chlorella* cultures in (●) sea water, (×) half-strength sea water, and (○) fresh water. A: . . . . ., *Stichococcus bacillaris* 379/1 e; - - - - -, *S. bacillaris* 379/1 c; ——, *S. 'cylindricus'* 379/5. B: . . ., *Chlorella stigmatophora*; - - - - -, *C.* 'Oslo' strain; ——, *C. ovalis.*

perhaps slightly better in half-strength sea water than in fresh water but much more slowly in full-strength sea water.

A similar experiment was done with five *Chlorella* strains—*C. stigmatophora* Butcher, Cambridge 211/20; *C. ovalis* Butcher, 211/21; *Chlorella* sp. from Oslo Fiord; *C. vulgaris* 211/11n and *C. pyrenoidosa* 211/8c; the two latter being freshwater strains. The three marine strains (Fig. 1B) show no strong preference for sea water against fresh water. Further investigation would be necessary to ascertain whether there are slight differences of growth due solely to salt concentration. The curves for *C. vulgaris* and *C. pyrenoidosa* showed no measurable growth in sea water and much less growth in half-strength sea water than in freshwater media.

Both these genera are as far as we know completely non-sexual. This implies that every surviving mutation starts a new clonal line and that the micro-evolution of these forms is entirely a divaricating system. In sexual organisms, on the other hand, there is a frequent recombination of characters within the population, the micro-evolutionary system is reticulate within the breeding group and pure lines are only obtained by artificial methods. Thus the species (or subspecies) in sexual forms is in general the potential breeding group, while in non-sexual forms there will be a large number of clonal lines in the conventional species. These clonal lines will perforce differ slightly from one another either physiologically, or morphologically, or both. In *S. bacillaris* differences of growth rate, tendency to form filaments and tendency to clump irregularly have been noted. To name each of these lines as a separate species would seriously overburden taxonomy, as practically every strain isolated seems slightly different and hence of a different clonal line. All that seems necessary in these forms is an aggregate species, perhaps with subspecies, and the clonal lines, where necessary, known by the reference numbers of a recognized culture collection. Thus it seems best to regard *Stichococcus bacillaris* as an (aggregate) species including many clonal lines all of which fit Naegeli's description and all of which grow well in fresh or sea water. With the *Chlorella* strains we have a more difficult problem because the taxonomy of the genus is in need of thorough investigation. However, there are small but constant differences and we may tentatively at least accept Butcher's species while noting that they are very unexacting about salinity.

SUMMARY

Several strains of *Stichococcus bacillaris* Naeg. and of the marine *S. cylindricus* Butcher were all found to fit morphologically in Naegeli's species. Both the marine and non-marine strains grow well in either fresh- or sea-water media. *S. cylindricus* must therefore be regarded as a synonym of *S. bacillaris*. Three marine *Chlorella* strains including *C. ovalis* Butcher and *C. stigmatophora* Butcher were also found to be quite unexacting about salinity. The significance

of small differences, particularly of growth rate, is discussed with regard to non-sexual organisms. In general, widely based species are advocated for such organisms, with the clonal lines, where appropriate, designated by the reference number of a recognized culture collection.

REFERENCES

Butcher, R. W., 1952. Contributions to our knowledge of the smaller marine algae. *J. mar. biol. Ass. U.K.*, Vol. 31, pp. 175–190.

Forest, H. S., 1954. Discussion of a portion of the Ulotrichaceae. *Castanea*, Vol. 19, pp. 61–75.

Fritsch, F. E., 1935. *Structure and Reproduction of the Algae*, Vol. 1. Cambridge University Press.

Naegeli, C., 1849. *Gattungen einzelliger Algen*. Zurich.

Prescott, G. W., 1951. *Algae of the Western Great Lakes Area*. Cranbrook Inst. Sci.

Smith, G. M., 1950. *Freshwater Algae of the United States*. New York: McGraw Hill.

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OBSERVATIONS ON THE RESISTANCE OF *TIGRIOPUS FULVUS* (FISCHER) TO CHANGES IN TEMPERATURE AND SALINITY

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(Text-figs. 1 and 2)

The harpacticid copepod *Tigriopus fulvus* (Fischer) has a wide distribution along the European coast, generally occurring in pools at or above high-water mark, where the environmental conditions are liable to sudden and extreme changes. Fraser (1936), while studying the life history and ecology of *T. fulvus*, has quoted Issel (1914): 'A few weeks of periodical observations of the pools since 1912 have drawn my attention to a phenomenon worth studying; as soon as the density of the water reaches a certain degree the copepod *T. fulvus* falls into a state of apparent death, from which it can awake even after a very long time and regain normal activity when the water is sufficiently diluted'. The experiments described in this paper were designed to investigate this phenomenon and other reactions of *T. fulvus* to changes in salinity and temperature.

EFFECTS OF SALINITY CHANGES

T. fulvus were collected from rock pools above high-water neaps at Port St Mary, Isle of Man, and transferred to sea water of salinity 34·0‰ in which they were conditioned for 2 days, to overcome the effects of the fluctuations in the salinity of the rock pools. Lower salinities were obtained by diluting the sea water with distilled water and higher salinities were obtained by adding Tidman's sea salt. The salinities were determined by titration with silver nitrate.

A graded series of twenty-three solutions was prepared with salinities ranging from 0·0 to 118·0‰. Approximately fifty specimens of conditioned *T. fulvus* were pipetted into 50 c.c. of each solution and left for a period of up to 15 days.

It was found that in distilled water the animals died after 84 h, while in solutions from salinity 4·2‰ up to 90·0‰ they were living normally after 15 days. In salinities above 90·0‰ *T. fulvus* fell into a state of apparent death, as described by Issel (1914); as soon as the animals were introduced into the solutions of salinity above 90·0‰ they ceased their activity and sank to the bottom of the dish and lay motionless. But when they were transferred back to sea water of salinity 34·0‰ they regained their normal activity after a period depending on the strength of the previous solution and the duration of immersion in it.

In order to investigate this behaviour a further series of experiments was conducted. For this a set of four solutions was made with salinities of 98·0, 135·0, 180·0 and 225·0‰ respectively. Fifty specimens of conditioned *T. fulvus* were transferred to each of the above solutions. At definite intervals animals were transferred from the high salinity solutions to sea water of salinity 34·0‰ and the time taken for complete recovery was noted. The temperature during the experiments varied between 16·0 and 18·0° C. The relation between the immersion period and the recovery period is shown in Fig. 1. The points plotted are the means of two experiments; variation between the repeat experiments was very small. It will be seen that the strength of the high salinity solutions had some effect on the period required to regain normal activity after transfer, those which were immersed in stronger solutions requiring a rather longer recovery period. This factor was, however, of much less importance than the immersion period, and in cases of immersion for more than 4 h may be virtually neglected. The species was found to be unable to survive indefinite immersion in any of the solutions in which the 'apparent death' response was observed, the survival period varying with the salinity.

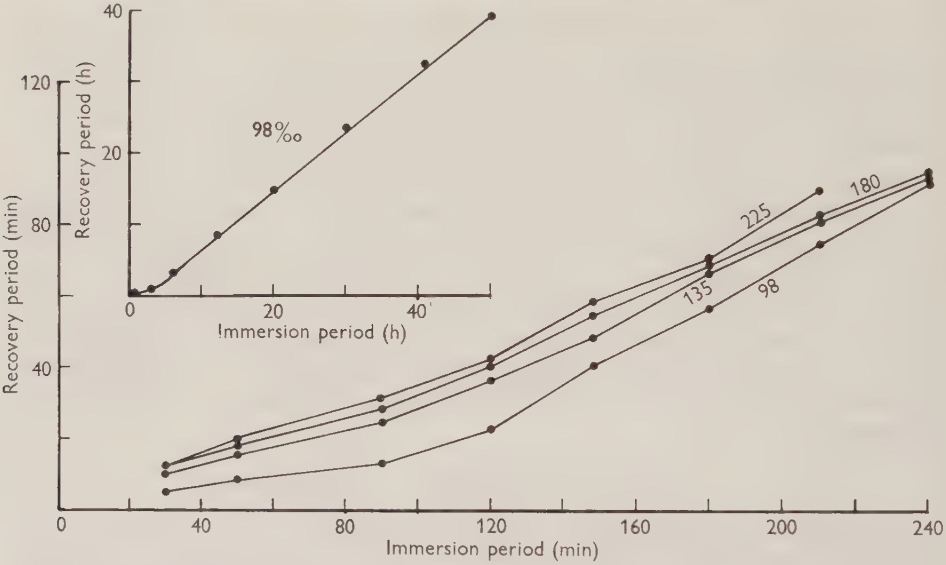

Fig. 1. *Tigriopus fulvus*. Relation between immersion period and the recovery period at salinities of 98, 135, 180, and 225‰, for various immersion periods up to 4 h. Inset: the same for a salinity of 98‰, when the immersion period was taken up to 50 h.

The maximum immersion periods, after which no recovery took place, were as follows:

Solution	Salinity (‰)	Maximum immersion period (h)
1	98	60
2	135	30
3	180	30
4	225	3

EFFECTS OF TEMPERATURE CHANGES

Two batches of twenty *T. fulvus* in 20 c.c. of sea water of salinity 34·0‰, immersed in a water-bath, were subjected to a slow rise in temperature at the rate of approximately 2° C per hour by means of a thermostat and heater. The animals behaved normally up to 34° C, but their movements became more rapid with increasing temperature until heat coma set in at 36° C when they became motionless and sank to the bottom of the dish. Death occurred when the temperature reached 38° C. The death-point was determined when no recovery took place after transferring the animals to room temperature (16° C) for a period of 24 h.

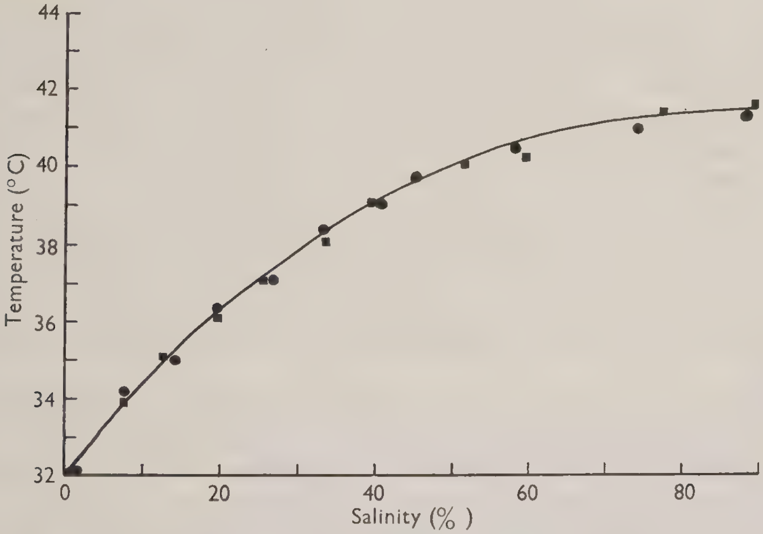

Fig. 2. *Tigriopus fulvus.* Lethal temperatures at different salinities. ■ First experiment, 18 August 1956. ● Second experiment, 26 September 1956.

In rock pools at high water, high temperatures are usually accompanied by increasing salinities on account of evaporation. An experiment, therefore, was set up to investigate the relation between the salinity of water and temperature tolerance of *T. fulvus*.

For this a series of eleven solutions of different salinities ranging from distilled water to 90·0‰ were made. Twenty specimens of *T. fulvus* were transferred from sea water to 20 c.c. of each solution in small dishes. These dishes were floated on a water-bath at 16° C and the temperature of the bath increased at a rate of approximately 2° C per hour. The *T. fulvus* were kept under continuous observation and the temperature of the water in each dish was taken at the 'death point'. This point was remarkably clear-cut, for about three-quarters of the animals always died simultaneously; the rest died within 3 min, during which the temperature never rose more than 0·1° C.

This experiment was repeated a month later with a fresh set of solutions and a new stock of *Tigriopus*. The second set of results agreed very closely with the first set, and both are shown in Fig. 2, which shows that the lethal temperatures varied over a range of 9·8° C depending on the salinity of the solutions. In distilled water death occurred at 32·0° C, while in salinity 90·0‰ the animals died at 41·8° C.

DISCUSSION

T. fulvus has been collected in pools at Port St Mary over a long period to serve as food for larval lobsters and post-larval fishes at the Marine Biological Station, Port Erin. It has been noticed that pools which had almost dried up, and which apparently had no *T. fulvus*, contained active specimens after rain even though the sea had not meanwhile covered the pools. I have also determined that the salinity and temperature fluctuations in the pools within 24 h could be from 5·0 to 30·0‰ and from 18° to 26° C respectively. The experiments on salinity tolerance show that *T. fulvus* can withstand very high salinities for appreciable periods by ceasing their activity and falling into the crevices of the rocks to tide over the drought period; this is obviously advantageous to animals inhabiting pools with violent fluctuations in salinity.

Salinity changes in the pools are correlated with changes in temperature; as the temperature goes up, evaporation causes the salinity to rise. The experiments show that with increasing salinity temperature tolerance also goes up; *T. fulvus* thus appears to be particularly well adapted to its habitat.

Observations on the pools, over a period of two months (June and July 1956) have shown that the fluctuations in salinity and temperature in the pools were from 5·0 to 42·0‰ and 13° to 27° C respectively. These changes are well within the tolerance limits of *T. fulvus* as shown in the foregoing experiments.

I am greatly indebted to Mr A. B. Bowers for his help in the collections and experimental work. I also wish to thank Mr J. S. Colman and Dr D. I. Williamson for their help in preparing the manuscript.

SUMMARY

Tigriopus fulvus can live normally within a salinity range of from 4·2 to 90‰.

In waters of salinities above 90‰ it falls into a state of apparent death from which it can recover if transferred back to lower salinities.

The relation between immersion period and the recovery period is only slightly different at different salinities.

The lethal temperatures vary between 32·0 and 41·8° C depending on the salinity of the sea water, the lethal temperature being higher in higher salinities.

The fluctuations in salinity and temperature in the pools where the animals were collected are well within their tolerance limits.

T. fulvus is well adapted to its habitat.

REFERENCES

Fraser, J. H., 1936. The occurrence, ecology and life history of *Tigriopus fulvus* (Fischer). *J. mar. biol. Ass. U.K.*, Vol. 20, pp. 523–536.

Issel, R., 1914. Vita latente per concentraz. dell'acqua e biologia delle pozze de scogliera. *Mitt. Zool. Sta. Neapel*, Bd. 22, pp. 191–255.

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THE RESPONSE OF THE LIMPET, *PATELLA VULGATA* L., TO WATERS OF DIFFERENT SALINITIES

By D. C. Arnold
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(Text-figs. 1–5)

During a study of the behaviour of the common British limpet, *Patella vulgata* L., undertaken in this laboratory, it was found that when dry this species reacts to splashing in a manner which can be correlated with the salinity of the water used. Since no experimental work relating to the chemosensitivity of the limpet could be traced in the available literature, these reactions were further studied in an attempt to identify the receptor regions responsible for detecting variations in salinity. The results so far obtained, while necessarily preliminary in nature, have indicated that in *Patella* the graded response to different salinities is mediated by receptors in the cephalic tentacles and mantle fringe.

MATERIALS AND METHODS

The limpets used in these experiments were obtained from bare or weed-covered surfaces at the East Rocks, St Andrews. They were removed by chipping away fragments of the rock on which they were lodged and any animals which appeared to have been damaged in the process were discarded. In the laboratory they were placed in running sea water for some hours, then left dry for about 8 h before the start of the experiments, most of which were performed during the morning or early afternoon of the day following collection. After use the limpets were kept for a further 3 or 4 weeks under circulation with prolonged periods of dryness each day; they survived well, moving and feeding with every appearance of normality.

On being brought into the laboratory, many of the limpets abandoned the rock to which they were attached and were then placed on the bases of inverted solid watch-glasses, to which the majority readily adhered. Those which did not leave the rock of their own accord were studied without being detached. Stimulation was provided by dropping about 2 ml. of water on to the apex of the shell, a small amount passing beneath the shell and affecting the animal. The response, a vertical movement of the front edge of the shell, was recorded by means of a heart lever (arm ratio 12:1). Further details are given in the accounts of the experiments concerned.

OBSERVATIONS AND EXPERIMENTS

THE RESPONSE TO SEA AND FRESH WATER

When dry, *Patella* remains quiescent, with only occasional slight upward movements of the shell, but, as upon the shore, the animal is not clamped hard down upon its support except when subjected to stimuli of a potentially dangerous nature. In this quiescent state the head is almost completely withdrawn into the mantle cavity, the foot spread over most of the surface beneath the shell and the fringe of the mantle extended almost, but not quite, as far as the edge of the shell (Fig. 1*a*).

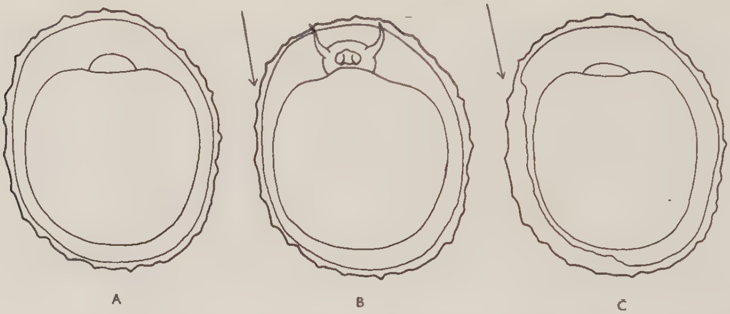

Fig. 1. The response of *Patella* to stimulation by sea and fresh water. A, ventral view of quiescent animal, drawn through glass; B, the same animal, after stimulation with sea water; C, the same animal after stimulation with fresh water. Arrows show direction of flow of water.

If a limpet in this condition was splashed with sea water, it responded by lifting the front edge of the shell, advancing the head and extending the cephalic tentacles up to or beyond the edge of the shell (positive response, Fig. 1*b*). The vertical movement of the shell was often considerable, being as much as 5 mm in a high-tide limpet of 50 mm length. The time elapsing between the stimulus and the commencement of the response varied from less than 30 sec to about 2 min. The response to a single stimulus was normally followed by a sharp contraction of the muscles and a return to the quiescent state. Under repeated stimulation the majority of limpets began to wander and once this had commenced would no longer give clear responses to splashing with sea water. The few animals encountered which gave no response to sea water were later found to have been damaged during or subsequent to collection.

Stimulation by splashing with fresh water elicited the opposite response. As the water flowed beneath the edge of the shell the mantle fringe contracted before it (Fig. 1*c*), the head was withdrawn even further into the mantle cavity than when the limpet was quiescent and the shell was clamped hard down to exclude the noxious liquid (negative response). Similar stimulation of limpets extended in response to sea water resulted in an immediate contraction, begun as soon as the water touched the body and completed in less than 30 sec. No animal failed to respond to fresh water in this manner.

SENSORY REGIONS RESPONSIBLE FOR THE REACTION

An inverted limpet will extend fully in an attempt to right itself, even if it is out of water at the time. This enables the body to be explored for the localization of sensory regions. Exploration by touch revealed five regions characterized by different local reactions to slight contact (Fig. 2). The sole of the foot (1) contracted at the point touched, leaving a deep pit. A similar light touch at the side of the foot (2) induced an outward movement which would ultimately bring the sole of the foot over the object touched. The body and the walls of the mantle cavity (3) were almost insensitive to touch, but the mantle fringe (4) and its tentacles were extremely sensitive and reacted to the slightest contact by immediate withdrawal. The head and cephalic tentacles (5) also recoiled sharply from contact with any object unfamiliar to the animal.

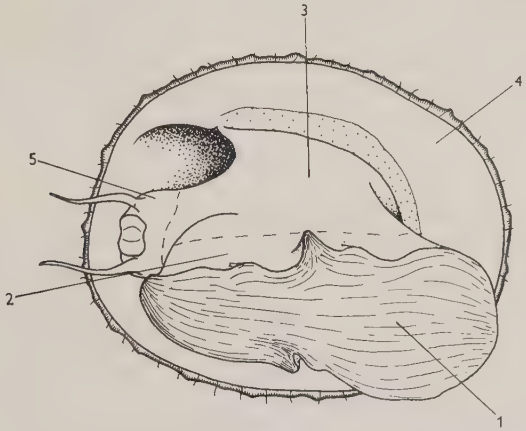

Fig. 2. Ventro-lateral view of extended limpet, showing major sensory regions. For explanation, see text.

A similar exploration was conducted by dropping water from a fine pipette on to these various regions. When sea water was used on a well-extended limpet regions 1–4 gave no recognizable local response, nor did the behaviour of the animal change. The cephalic tentacles, however, would explore the surface of the pipette, if this could be reached, withdrawing only when the pipette ran dry. The foot and general body surface proved equally insensitive to contact with fresh water, but both the mantle fringe and the cephalic tentacles contracted sharply when stimulated in this manner.

While an inverted limpet is endeavouring to right itself it is easy to remove almost all the sea water from the mantle cavity without affecting the activity of the animal. Fresh water dropped into the drained cavity was found not to elicit a response, provided that neither the mantle fringe nor the cephalic tentacles were touched in the process. Only when the cavity had been completely filled with fresh water did the activity of the limpet slow and the head withdraw into the cavity. This contraction did not have the appearance of a response to stimulation, but seemed rather a general 'malaise'.

The osphradia, for which both chemoreceptive and tactile functions have been proposed (Hulbert & Yonge, 1937), merited special attention, but, owing to their position far back in the floor of the mantle cavity, could not be removed from intact limpets, while shell-less animals, though they lived, remained inactive. Attempts to destroy the osphradia by cautery were also unsuccessful and therefore it could not be determined directly whether these organs were in fact sensitive to the stimuli used. However, close observation from below of limpets attached to glass, aided by the addition of carmine to the stimulating liquid, showed clearly that the responses were obtained without the entry of water into the mantle cavity at all. The response of *Patella* to sea and fresh water is thus not mediated by the osphradia.

THE RESPONSE TO DILUTED SEA WATER

On the shore *Patella* is subject to considerable variation of salinity. In order to determine the tolerance to dilution limpets were collected from six levels on the shore and their responses compared. The tide levels used (H.W.O.N.T., H.W.E.N.T., M.T., L.W.E.N.T., L.W.O.N.T. and a little above L.W.O.S.T.) were determined partly by algal zonation and partly by direct observation during collection. At each tide level specimens were chosen within two size ranges, *c.* 25 and *c.* 45 mm. length, in order to determine whether or not there was a difference in response ascribable to age. In fact, no difference was found.

Each animal was tested with a range of salinities produced by diluting sea water in 10% steps with fresh water. As 'fresh' water, rain, tap and distilled water were used, but in parallel experiments gave similar results. Stimuli were given at approximately 5 min intervals and were at random, with the exception that the same stimulus was not given twice in succession. After each stimulus the limpet was washed briefly with fresh water. If a response was given its magnitude was determined by measuring the height of the trace in respect to a superimposed base-line determined from the positions of maximum contraction at the beginning and end of the experiment. Animals on glass frequently moved slightly during the course of an experiment, and when wandering took place to any great extent that sequence of observations was rejected. If no positive response was given the stimulus was marked as 0, irrespective of whether the limpet did or did not contract to the maximum extent.

All limpets tested were tolerant to some at least of the dilutions, and the magnitude of their responses was found to correspond in fair degree with the salinity used. The responses of a given animal to similar stimuli were quite consistent (Table 1), though there was a tendency to greater variability as the salinity decreased. The largest responses were invariably given to undiluted sea water, but in several animals the size of the response did not fall off regularly with dilution, but showed an additional secondary peak. The reason for this is unknown. Comparison of limpets taken from different tide levels showed that while there was individual variation within each group, especially obvious in those from the mid-tide region, several distinct trends could easily be recognized when passing from the upper to the lower tide limits. Limpets taken from H.W.O.N.T. (the upper limit of colonization) gave very consistent and clear-cut responses and were tolerant to salinities below 50% sea water (Fig. 3) and in one case even as low as 20%. Their movements were quick to commence, large and smooth. In contrast, limpets collected a little above L.W.O.S.T. (the lower limit of colonization) were negative to salinities of 80% sea water or less and gave much smaller and more irregular responses than did high-tide animals of similar size. These extremes were linked by the range of behaviour of animals drawn from intervening tide levels (Fig. 4).

TABLE 1. VERTICAL MOVEMENT IN MM OF THE FRONT EDGE OF THE SHELL OF TWO LIMPETS FROM DIFFERENT TIDE LEVELS IN RESPONSE TO SPLASHING WITH NORMAL AND DILUTED SEA WATER

Tide level	Length of shell (mm)	Relative salinity of sea water						
		100%	90%	80%	70%	60%	50%	40%
H.W.O.N.T.	26	2·00	1·33	1·25	0·83	0·58	0·25	0
		2·00	1·58	1·17	0·75	0·50	0·17	0
		1·91	1·75	1·42	1·25	1·25	0·42	0
		2·09	—	—	—	—	—	—
	Mean	2·00	1·55	1·28	0·94	0·78	0·28	0
L.W.E.N.T.	24	1·75	1·42	1·17	1·50	0·92	0·25	—
		2·00	1·25	1·08	1·42	1·17	0	—
		1·75	1·58	1·42	1·42	0·75	0	—
	Mean	1·83	1·42	1·22	1·45	0·95	0·08	—

THE RESPONSE TO SOLUTIONS OF SODIUM CHLORIDE

Some limpets from H.W.O.N.T. were tested with solutions of sodium chloride. This was of Analar grade made up in distilled water in concentrations ranging from 100 g/l. down to 20 g/l. The responses given to these solutions resembled those given to natural and diluted sea water, except that the movements were more jerky and less rapid, while the limpets soon became refractory. Consistent results could only be obtained by allowing the animals frequent and lengthy periods for recovery during the course of each experiment.

Responses to undiluted sea water and to a sodium chloride solution of equivalent salinity were of similar magnitude, while abnormally large responses

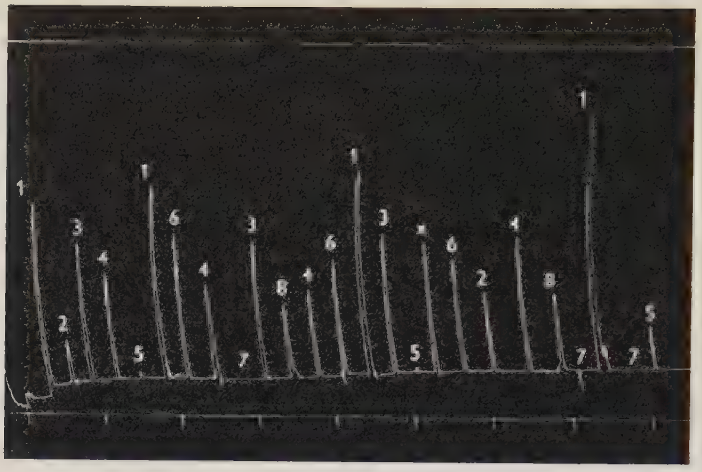

Fig. 3. Response of limpet, taken from H.W.O.N.T., 46 mm long, to normal and diluted sea water. Time at 15 min intervals. *Key to numbering*: 1 = normal sea water; 2 = 60 % sea water; 3 = 90 % sea water; 4 = 70 % sea water; 5 = 40 % sea water; 6 = 80 % sea water; 7 = 30 % sea water; 8 = 50 % sea water. × = spontaneous movement.

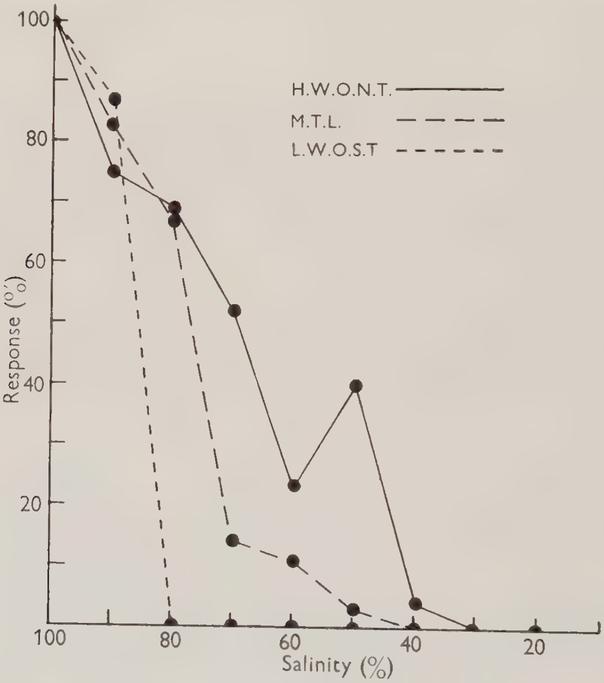

Fig. 4. The response of limpets from different tide levels to various salinities. Salinities expressed as percentages of undiluted sea water, responses as percentages of response to undiluted sea water.

were given to solutions containing 40g/l. NaCl (Fig. 5). Above this concentration the responses once more diminished in size.

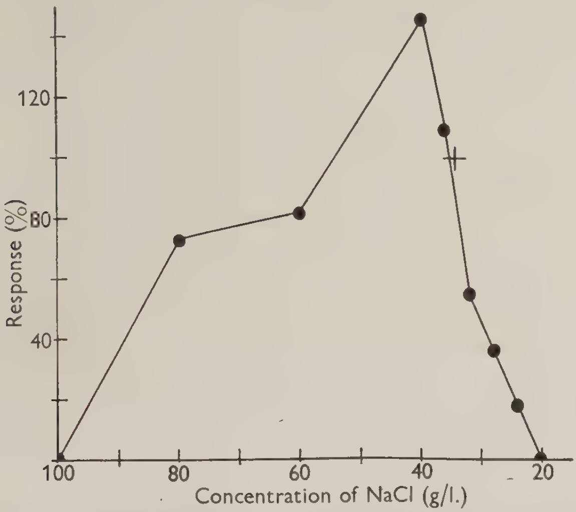

Fig. 5. The response of a limpet from H.W.O.N.T. to various NaCl concentrations. Concentrations expressed as g NaCl/l., responses as percentages of response to undiluted sea water (salinity *c.* 34·5 ‰), the position of which is shown by a cross.

DISCUSSION

These experiments have yielded the results which are to be expected on *a priori* considerations. The negative response of *Patella* to water of low salinity, the increasing tolerance as one ascends the shore and the more immediate and larger responses to splash shown by the high-tide limpets, are all characters which are to be expected as adaptations to a progressively more rigorous environment in which activity is curtailed by ever longer periods of dryness. Whether these potentialities are possessed by all limpets and expressed according to their position on the shore, or whether the possession of certain powers of activity under adverse conditions determines the position ultimately occupied by the adult limpet is not known.

The perception of salinity by the mantle fringe and cephalic tentacles is also to be expected, since these are the parts of the animal which are most exposed to the environment.

SUMMARY

Patella vulgata gives a positive response to splashing with sea water or water of high salinity, and a negative response to fresh water or water of low salinity. The intensity of the positive response broadly corresponds with the salinity of the water used. There is a greater response to splash and a greater tolerance

to reduced salinity in limpets from high-tide than in those from low-tide regions. A positive response is given to NaCl solutions of concentrations approximately equivalent to sea water, while a supra-normal response is given to concentrations somewhat above that of sea water. The perception of salinity is by receptors in the cephalic tentacles and mantle fringe.

REFERENCE

Hulbert, G. C. E. B. & Yonge, C. M., 1937. A possible function of the osphradium in the Gastropoda. *Nature, Lond.*, Vol. 139, pp. 840–1.

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CHROMOSOME NUMBERS OF GAMMARIDS

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(Plate I and Text-figs. 1–4)

The taxonomy of gammarid amphipods is a notoriously difficult subject. The group comprises a large number of recognized species whose identification depends on an expert knowledge of morphological minutiae. We began the study to be described in the present paper with the hope that a knowledge of the chromosome numbers of gammarids might be of taxonomic value. This hope has proved in the event to be largely ill-founded. We also undertook the present study in order to resolve a dispute as to whether polyploidy has played a part in gammarid evolution. This question has been clearly settled in the negative sense.

The nuclear cytology of most Crustacea presents severe technical problems. All crustaceans so far examined have relatively small, often extremely small, chromosomes. Most of the higher Crustacea have high chromosome numbers, those of decapods being the highest chromosome numbers in the whole animal kingdom. Gammarids, with small chromosomes and diploid numbers up in the fifties, are by no means easy cytological material.

From the literature describing previous work on gammarid cytology it was abundantly clear that we would make little progress using stained paraffin sections of testes of the various species. Aceto-carmine-stained squash preparations of gammarid testes have been used by the most reliable previous workers, but in our limited experience such preparations are frequently defective as regards quality of fixation and, critically and regularly, in the absence of analysable side views of first meiotic metaphase. Staiger & Bocquet's paper demonstrating female heterogamety in certain isopods (Staiger & Bocquet, 1954) suggested to us that it might be more profitable to base our cytological study of gammarids on oocyte meiosis. By making squash preparations of oocytes just prior to oviposition Staiger & Bocquet obtained analysable side views of first meiotic metaphase, and it is clear from their illustrations that the chromosomes of *Jaera* oocytes are considerably larger than those of spermatocytes. There are comparable advantages in studying oocyte as opposed to spermatocyte meiosis in the prosobranch gastropod *Nucella lapillus* (L.) (Staiger, 1954, as *Purpura*) and the earthworm *Eisenia foetida* (Savigny) (Callan, unpublished).

Previous determinations of the chromosome numbers of gammarids are listed by Le Calvez & Certain (1951). Their list, with minor modifications, is reproduced in Table 1. Within this list the haploid numbers of 13 for *Gammarus chevreuxi* and *G. duebeni*, on the authority of Palmer and Le Roux,[1] respectively, stand apart from all the other determinations, which range from $n=22$ to $n=27$. At first sight this incongruity might appear to indicate that *G. chevreuxi* and *G. duebeni* are diploid members of a predominantly tetraploid group. However, Le Calvez & Certain (1951) redetermined the chromosome numbers of *G. chevreuxi* and *G. duebeni* (specimens of the latter species from the same locality as those with which Mme Le Roux had previously worked), and found haploid numbers of 26 and 27. With considerable justification they suggested that both Palmer's and Le Roux's determinations were based on inadequate techniques and were erroneous. Nevertheless, some uncertainty still remained: Palmer's *G. chevreuxi* material originated from Plymouth, whereas Le Calvez & Certain's *G. chevreuxi* came from Roscoff, and thus the alternative chromosome numbers might conceivably reflect genuine interracial differences.

TABLE 1. PUBLISHED CHROMOSOME NUMBERS OF GAMMARIDS

Species	Haploid number	Authority
Gammarus chevreuxi Sexton	*ca.* 22	Huxley, 1923
G. chevreuxi Sexton	13	Palmer, 1926
G. chevreuxi Sexton	26	Le Calvez & Certain, 1951
G. duebeni Lilljeborg	13	Le Roux, 1933*
G. duebeni Lilljeborg	27	Le Calvez & Certain, 1951
G. zaddachi Sexton	26	Le Calvez, 1949†
G. pulex pulex (L.)	26	Poisson & Le Calvez, 1948
G. locusta (L.)	26	Poisson & Le Calvez, 1948
G. pungens Milne Edwards	24	Le Calvez & Certain, 1951
G. annandalei Tattersall	27	Niiyama, 1935
Marinogammarus marinus (Leach)	26	Le Calvez & Certain, 1951
Echinogammarus berilloni (Catta)	26	Le Calvez, 1949
Carinogammarus roeseli (Gervais)	26	Le Calvez, 1949
Niphargus plateaui var. *elongatus* Chevreux	25	Le Calvez, 1949
N. tatrensis var. *lunzensis* Schell.	25	Le Calvez, 1949
Melita palmata (Montagu)	22	Le Calvez, 1949
Maera othonis (Milne Edwards)	23	Le Calvez & Certain, 1951
M. grossimana (Montagu)	23	Le Calvez & Certain, 1951
Metacrangonyx longipes Chevreux	26	Le Calvez & Certain, 1951

* In all probability Le Roux's species was really *Gammarus z. zaddachi*.

† *G. zaddachi* was incorrectly designated *G. duebeni* in Le Calvez's 1949 paper: the error was corrected in the 1951 paper by Le Calvez & Certain.

By working with oocytes we have been able to make accurate chromosome counts in nine gammarid species. However, since in at least three of these species chromosome number varies from individual to individual, and since the chromosome numbers of different species overlap, it is clear that chromosome counts will not be of great value to students of gammarid systematics.

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[1] According to G. M. Spooner (personal communication) the illustrations and comparative measurements of limbs given in Le Roux's paper indicate that she was dealing with the typical subspecies of *Gammarus zaddachi* Sexton and not with *G. duebeni*.

Reliance can be placed on the identification of material which we have studied cytologically since we have enjoyed the invaluable assistance of two experts on gammarid taxonomy, Dr C. Edwards of the Scottish Marine Biological Association at Millport, and Mr G. M. Spooner of the Marine Biological Association of the United Kingdom at Plymouth.

MATERIALS AND METHODS

We started our cytological work on gammarids by making aceto-orcein squash preparations of fresh and of acetic-alcohol prefixed testes of several species. In well-flattened preparations equatorial plates of first meiotic metaphase are only to be seen in polar view: the bivalents are crowded so close together (the first metaphase equatorial plates of *G. chevreuxi*, *G. locusta* and of *Marinogammarus marinus* are only some 7 or 8 μ in diameter) that it proved quite impossible to make accurate chromosome counts. At second meiotic metaphase the difficulties are still greater: in *Gammarus chevreuxi*, for example, the diameter of a second metaphase equatorial plate is only some 4–5 μ. Occasional spermatogonial prometaphases can be counted with fair accuracy, but in most preparations these are not sufficiently frequent to allow of adequate cross-checking. In short, we would not have been able to establish with certainty the existence of chromosomal polymorphism in gammarid species by working with testis preparations alone.

In order to work on oocyte meiosis it is necessary to know the state of maturation of the eggs relative to the macroscopic cycle of events connected with oviposition. As is well-known, male and female gammarids associate in precopula several days before egg-laying. The female moults, and immediately thereafter the male turns the female round so that the pair lie transverse to one another: the male then ejects sperm into the female's brood-chamber. The pre-oviposition moult is an event which can be readily observed and it forms a useful reference point within the maturation cycle. Thirty to ninety minutes after the moult, dependent on species and on temperature, the first eggs drop down the paired oviducts into the brood-pouch. In pale species with well-pigmented eggs, e.g. *Marinogammarus pirloti*, this event can also be readily observed. The eggs collect in the brood-pouch in two thin-walled transient structures, the so-called 'mucous sacs', which slowly dissolve and disappear completely some 2 hr later: sperm penetration takes place at this time.

We have established the time-scale of the cytological events taking place during maturation of the eggs in *Gammarus pulex* L., the animals being maintained in good conditions at a constant temperature of 15° C. This time-scale is shown in Table 2.

Chromosome numbers can be established with certainty at three stages: first and second meiotic metaphase, and first cleavage metaphase (later cleavage metaphases are also of value, though they become progressively more difficult to analyze the older the embryo). Typical photographs of these three stages are shown in Pl. I, figs. 2–5; for the sake of comparison a photograph of a particularly clear *G. pulex* spermatocyte first meiotic metaphase is also included in this plate (fig. 1). The great superiority of the egg preparations is evident. Of these three stages, oocyte first meiotic metaphase is the most informative: unfortunately it presents greater technical difficulties than do second meiotic metaphase or first cleavage mitosis in that the eggs in oviduct and ovary are very fragile, they stick together and cannot be entirely freed from ovarian wall tissue, and the stage is of short duration. We have intentionally worked with first meiotic metaphase only in *G. pulex* (first meiotic metaphase of *Marinogammarus marinus* was obtained by accident). Studies on the other species have been confined to second meiotic metaphase, in several respects the most easy stage with which to work.

TABLE 2. *GAMMARUS PULEX* (L.): TIME-SCALE OF CYTOLOGICAL EVENTS AT 15° C

Time from pre-oviposition moult of female (min)	Time from oviposition (min)	Stage
15	—	Diplotene
30	—	Diakinesis
45–60	—	Prometaphase I
75	0	Metaphase I
90	15	Anaphase I
100	25	Telophase I
110	35	Prometaphase II
115–195	40–120	Metaphase II
210	135	Anaphase II
225	150	Telophase II: sperm penetration
225–325	180–25	Approximation of pronuclei
425–525	350–450	Metaphase of 1st cleavage

For preparations of first meiotic metaphase in *Gammarus pulex* isolated pairs in precopula were watched for moulting. Sixty to ninety minutes after moulting the female was removed, slit open along its dorsal side and fixed entire in Carnoy's fluid (three parts absolute alcohol: one part glacial acetic acid). Soon thereafter the ovaries and oviducts were dissected out and transferred to fresh Carnoy's fixative for 12–18 h.

For preparations of second meiotic metaphase in all species maintained at a temperature of 15° C. the coupled pairs were watched for moulting and for the subsequent start of oviposition. Two hours after the beginning of oviposition the eggs were dissected out from the brood pouch and mucous sacs, washed in sea or tap water as appropriate, fixed in Carnoy's fluid, transferred to fresh fixative a few minutes later, and left for 12–18 h. For preparations of embryonic mitoses the embryos were similarly treated, being however allowed to remain for longer periods in the female's brood pouch prior to fixation.

After fixation the ovaries, eggs or embryos were transferred with as little fixative as possible to a freshly filtered 1 % orcein solution made up in 45 %

acetic acid and there left to stain for 4–6 h. Natural orcein gave us considerable trouble since it forms a noticeable precipitate within a few hours. Synthetic orcein supplied by G. T. Gurr later proved to be much superior both in staining qualities and freedom from precipitate.

The stained material was arranged on a clean coverslip over which an albuminized slide was subsequently lowered. Between folded filter-papers slide and coverslip were squashed very firmly together, and the edges of the coverslip then sealed with paraffin wax.

Most of the observations were made on such temporary preparations, which keep in good condition for several weeks if stored in a refrigerator just above freezing-point. Valuable preparations were later made permanent. The wax was soaked off in xylol and the preparation inverted in a ridged dish containing 90% alcohol. After a day or two in 90% alcohol such coverslips as had not separated freely from their slides were prized off with a razor blade. The material generally remained attached to the albuminized slide, which was then dehydrated in absolute alcohol and mounted in Euparal.

Gammarus pulex (L.)

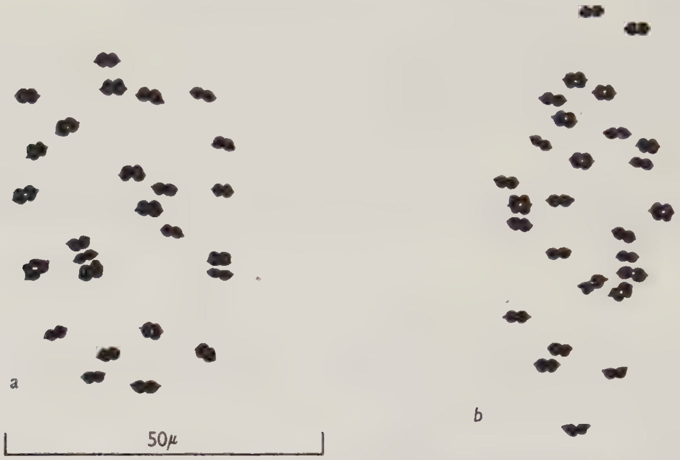
Although we did not start our study of gammarid chromosomes with the freshwater *Gammarus pulex*, it is convenient to deal with this species first. Our material was supplied by the Freshwater Biological Association, Ambleside.

Chromosome numbers found in oocytes at second meiotic metaphase are shown in Table III. From this table it will be seen that five females produced oocytes each containing 26 chromosomes, two females produced equal numbers of oocytes with 26 or 27 chromosomes, and one female produced oocytes each containing 27 chromosomes. Characteristic second meiotic metaphase chromosome groups, one with 26 and the other with 27 chromosomes, are shown in Text-fig. 1 and in Pl. I, figs. 3 and 4.

These observations suggested that *G. pulex* may have alternative diploid chromosome numbers of 52, 53 or 54. Our attention was now directed towards mitotic divisions in embryos. The existence of 52- and 53-type embryos was confirmed, though no 54-type was encountered amongst forty-four embryos from twelve females. 53-type embryos were present amongst the egg-batches of five different females, but since we do not know whether any of these egg-batches were uniformly of 53-type the chromosome constitutions of the parents, male or female, cannot be certainly stated.

We next examined first meiotic metaphase in oocytes, and for this purpose fixed the ovaries of some eighty females at the appropriate stage. Amongst these females six were definitely established as 53-type and three as 54-type. The majority of the remaining females were certainly 52-type, but the absolute ratio is not worth quoting since the constitution of too many animals within the group could not be definitely established.

In 52-type females the oocytes regularly show 26 bivalents: *G. pulex* chromosomes have median or submedian centromeres, and most of the bivalents appear to be associated by single chiasmata in both arm pairs (Pl. I, fig. 2; Text-fig. 2*a*, *b*). In 53-type females the oocytes regularly show 26 bivalents and a single univalent (Text-fig. 2*c*, *d*). Trivalent associations were not observed in any of the sixty-two cells of this type which were fully analysed. In 54-type females the oocytes generally show 27 bivalents (Text-fig. 2*c*, *f*), though in four cells out of the twenty-seven of this type which were fully analyzed there was some uncertainty as to the status of one pair of chromosomes, which had either disjoined precociously or, less probably, which had failed to associate earlier in meiosis. No multivalent chromosome associations were observed in oocytes from 54-type females.

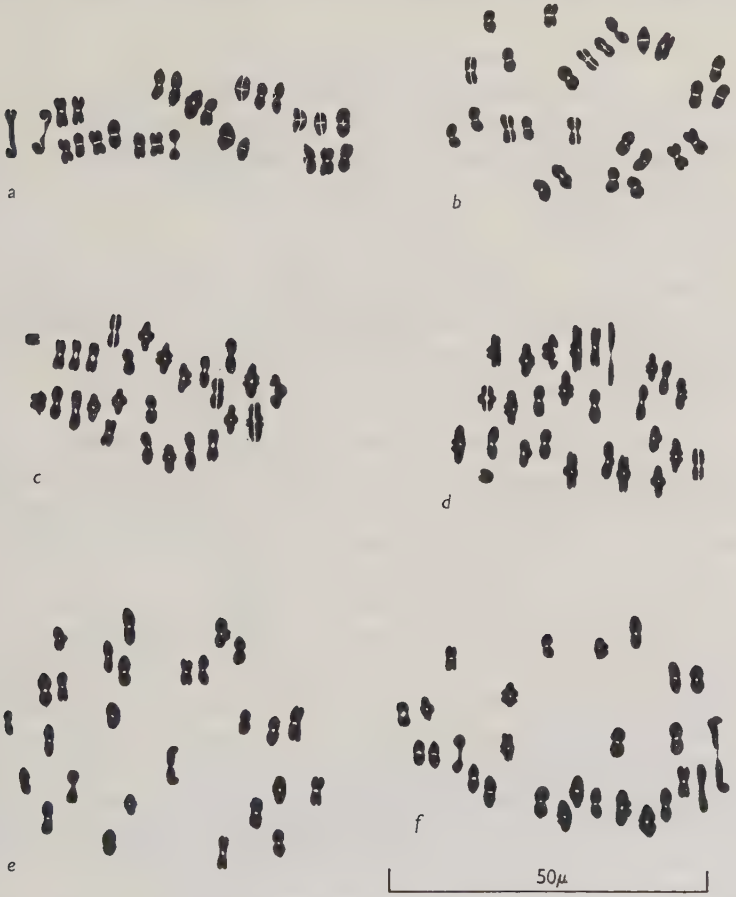


Text-fig. 1. Camera lucida drawings of second meiotic metaphase chromosome groups from oocytes of *Gammarus pulex*: (*a*) with 26 and (*b*) with 27 chromosomes.

TABLE 3. CHROMOSOMES OF *GAMMARUS PULEX* (L.)

Specimen number	Number of secondary oocytes with chromosome numbers of 26	27	Assessed diploid chromosome number
5	8	10	53
7	12	—	52
9	—	16	54
10	6	7	53
11	8	—	52
12	6	—	52
15	5	—	52
29	5	—	52

To judge from the chromosome counts made at second meiotic metaphase, chromosome disjunction at first anaphase is regular in 52- and 54-type oocytes, whilst in 53-type oocytes the univalent appears normally to pass undivided into secondary oocyte or polar body with equal frequency. The picture is complicated, however, by the occasional presence in 52- 53- or 54-type females of minute fragment chromosomes which, at least on occasion, lag behind on the equator at first anaphase. We do not know the fate of such fragment chromosomes nor their relationship to the chromosomes of the regular complement. They are not the outcome of crossing-over within inversion heterozygotes since they are not accompanied by bridges and, when present, are to be seen in every oocyte of a particular female.



Text-fig. 2. Camera lucida drawings of first meiotic metaphase chromosome groups from oocytes of *Gammarus pulex*: (*a*) and (*b*) with 26 bivalents; (*c*) and (*d*) with 26 bivalents plus one univalent (top left in *c*, bottom left in *d*); (*e*) and (*f*) with 27 bivalents.

The chromosomes of *G. pulex* are considerably larger than those of the other gammarids which we have studied and from a cytological standpoint this is not a difficult species with which to work. It would be of interest to study the chromosome constitutions of different populations of *G. pulex* and also to determine whether, since in this animal several generations succeed one another within the year, there is any seasonal variation in chromosome constitution within a given population.

Gammarus duebeni Lilljeborg

A few oocytes from four examples of this species collected at the East Rocks, St Andrews, gave chromosome counts of 27 at second meiotic metaphase. This number agrees with the determination of Le Calvez & Certain (1951) and it corroborates their suggestion that Madame Le Roux's determination was erroneous.

Gammarus locusta (L.)

A few oocytes from six examples of this species supplied by the Dove Marine Laboratory, Cullercoats, gave chromosome counts of 26 at second meiotic metaphase. This number agrees with the determination of Poisson & Le Calvez (1948).

Gammarus zaddachi Sexton subsp. *salinus* Spooner

A few oocytes from two examples of this species collected at the East Rocks, St Andrews, gave chromosome counts of 26 at second meiotic metaphase. This number again agrees with the determination of Le Calvez (1949).

Gammarus chevreuxi Sexton

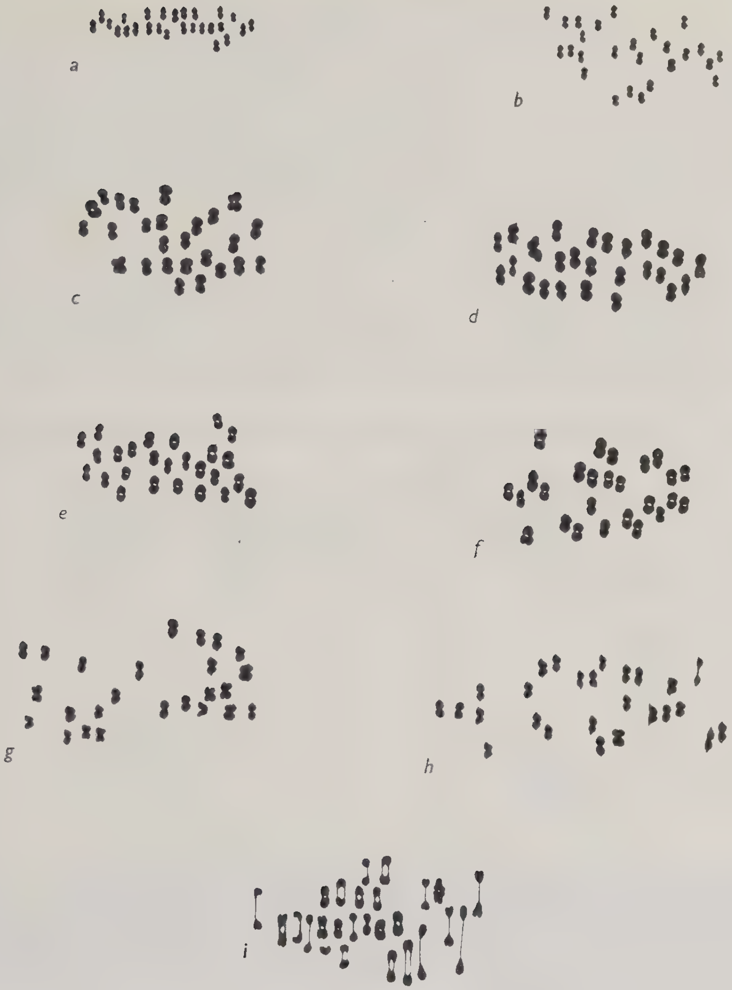
Specimens of *G. chevreuxi* from Chelson Meadow, Plym Estuary, were supplied by the Marine Biological Association's laboratory at Plymouth and were thus from the same site as those which Palmer (1926) studied. Forty oocyte second meiotic metaphase chromosome groups from ten females were counted and a constant haploid number of 26 established. As can be seen in Text-fig. 3 *a* and *b*, the chromosomes of *G. chevreuxi* are very small indeed. The determination of Le Calvez & Certain (1951) has been corroborated, and their suggestion that Palmer's determination was erroneous upheld.

Marinogammarus obtusatus (Dahl)

This species was collected at the East Rocks, St Andrews. Eighty-nine oocyte second meiotic metaphase chromosome groups from fifteen females were counted and a constant haploid number of 26 established. (See Pl. I, fig. 6; Text-fig. 3 *c*, *d*).

Marinogammarus finmarchicus (Dahl)

This species was collected at the East Rocks, St Andrews. Twenty-two oocyte second meiotic metaphase chromosome groups from four females each gave counts of 26 (see Text-fig. 3 *e*, *f*).



Text-fig. 3. (*a*) and (*b*) second meiotic metaphase chromosome groups from oocytes of *Gammarus chevreuxi*, both with 26 chromosomes. (*c*) and (*d*) second meiotic metaphase chromosome groups from oocytes of *Marinogammarus obtusatus*, both with 26 chromosomes. (*e*) and (*f*) second meiotic metaphase chromosome groups from oocytes of *M. finmarchicus*. both with 26 chromosomes. (*g*) and (*h*) second meiotic metaphase chromosome groups from oocytes of *M. marinus*, (*g*) with 25 and (*h*) with 26 chromosomes. (*i*) first meiotic metaphase chromosome group from oocyte of *Marinogammarus marinus* with 25 bivalents plus one univalent. All same magnification as Text-fig. 1.

Marinogammarus marinus (Leach)

This species was collected at the East Rocks, Eden Estuary and Harbour, St Andrews. Apart from one exceptional observation on a delayed first metaphase, the chromosomes of this species were studied at first meiotic anaphase and second meiotic metaphase: the counts are listed in Table 4.

The chromosomes of *M. marinus* (Text-fig. 3*g*, *h*) are nearly as small as those of *G. chevreuxi*, and a great many preparations could not be analysed. However, sufficient were scored to establish that this species shows a chromosomal polymorphism similar to that of *G. pulex*, though with alternative diploid numbers of 50, 51 and 52. By good fortune specimen 33 contained one oocyte with a delayed and well-spread first meiotic metaphase, and this preparation showed 25 bivalents plus one univalent (Text-fig. 3*i*). Of the ten females studied four were 50-type, five were 51-type and one 52-type.

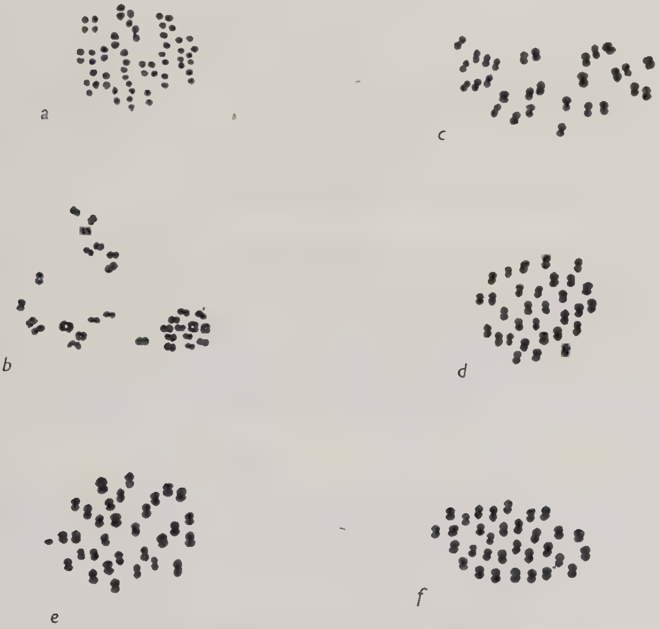
TABLE 4. CHROMOSOMES OF *MARINOGAMMARUS MARINUS* (LEACH)

Specimen number	Numbers of secondary oocytes with chromosome numbers of		Numbers of first polar bodies with chromosome numbers of		Assessed diploid chromosome number
	25	26	25	26	
5	—	10	—	—	52
23	3	3	—	—	51
33	1	2	2	1	51
36	1	1	1	1	51
38	2	3	—	—	51
39	5	—	5	—	50
40	2	2	—	—	51
41	3	—	—	—	50?
43	2	5	—	—	51
44	10	—	—	—	50

Marinogammarus pirloti Sexton & Spooner

This species was collected at the East Rocks, St Andrews, in a region where fresh water drains down over the tidal zone. *M. pirloti* happened to be the first gammarid species which we studied, and it presents a cytological problem of some complexity.

By virtue of the pale coloration of the females and the intense brown or black pigmentation of the eggs, the species provides admirable material for following the cycle of events at reproduction. Unfortunately, however, its chromosomes are as small as those of *M. marinus*, and there are more of them (Text-fig. 4). Counts were made at second meiotic metaphase in secondary oocytes (Pl. I, figs. 7, 8), and the species is exceptional in that at this stage the polar body chromosomes are still condensed and also frequently countable. The counts are summarized in Table 5, numbers in brackets being counts made on polar body chromosome groups. It will be seen that in the six individuals studied haploid numbers range from 29 to 32 and assessed diploid numbers from 59 to (?) 63. The assessed diploid numbers could only be established when complementary oocyte and polar body chromosome groups were both countable (Text-fig. 4*a*, *b*), and even in such cases there were sometimes grounds for uncertainty. To judge from the appearance of some second meiotic metaphase chromosome groups, notably two of 32 in specimen 15 and one of 32 in specimen 19, chromosomes present as univalents during first meiosis may on occasion divide equationally at first anaphase. The normal second metaphase chromosomes are dumb-bell shaped, the constriction



Text-fig. 4. (*a*) Early second meiotic anaphase and (*b*) first polar body chromosome groups from oocytes of *Marinogammarus pirloti*: (*a*) with 30 × 2 chromosomes; (*b*) with 29 chromosomes. (*c*), (*d*), (*e*) and (*f*) second meiotic metaphase chromosome groups from oocytes of *M. pirloti*: (*c*) with 29 chromosomes; (*d*) with 31 chromosomes; (*e*) with 32 chromosomes, the one on the extreme left being the product of the equational division of a univalent during first meiosis; (*f*) with 32 chromosomes. All same magnification as Text-fig. 1.

TABLE 5. CHROMOSOMES OF *MARINOGAMMARUS PIRLOTI* SEXTON & SPOONER

Specimen number	Numbers of secondary oocytes (or 1st polar bodies) with chromosome numbers of				Assessed diploid chromosome number
	29	30	31	32	
1	—	2	1	—	61?
2	—	2	2 (1)	—	61
3	5 (2)	6 (2)	—	—	59
15	—	1	—	3 (1)	62
18	—	—	3	4	63?
19	2	2 (1)	2 (1)	2 (1)	61

marking the centromere: it is suggested that the small chromosome lacking a constriction shown in Pl. I, fig. 8, and in Text-fig. 4*e*, is the product of such an equational division.

It would clearly be of interest to work on the first meiotic division of *M. pirloti* and to carry out a much more extensive cytological survey. The species unquestionably shows chromosomal polymorphism, and higher chromosome numbers than any previously recorded for gammarids.

DISCUSSION

From the observations recorded in this paper there would appear to be no further grounds for supposing that polyploidy has played a part in gammarid evolution. In support of the earlier observations of Le Calvez & Certain (1951), the haploid chromosome numbers of *Gammarus duebeni* (27) and of *G. chevreuxi* (26) have been shown to fall within the range of other gammarid haploid numbers.

From what we have seen of gammarid chromosomes it seems most unlikely that chromosome studies will be of material assistance to taxonomists working on this group. The chromosomes, even in oocytes, are generally small, and the numbers overlap from species to species. Moreover, we have established that chromosome number, far from being a well-defined taxonomic character, is variable in at least the three species *G. pulex*, *Marinogammarus marinus* and *M. pirloti*.

Apart from the special case of sex-determination, variable chromosome numbers may be due to the existence of a Robertsonian 'fragmentation-fusion' system (Robertson, 1916), where the two arms of V-shaped chromosomes may alternatively be represented by single-armed rod-shaped chromosomes, as within and between grasshopper species, and in the case of the prosobranch gastropod *Nucella lapillus* (L.) (Staiger, 1954, as *Purpura*); or they may be due to the presence of supernumeraries extra to a basic complement. The variable chromosome numbers of gammarids do not appear directly related to a sex-determining mechanism, though it is certainly conceivable that they may in some fashion be indirectly related. Clear proof of a Robertsonian system in gammarids is also wanting. In such a system the heterozygotes generally show trivalents at first meiosis where V-shaped chromosomes are associated with their genetically related two rod-shaped chromosomes, and indeed balanced disjunction within such a system is only possible when in heterozygotes trivalent associations are formed with great regularity. No multivalent associations were observed throughout our reasonably extensive studies on *Gammarus pulex*.

It is therefore reasonable to assume that the variable chromosome numbers of gammarids are due to the presence of supernumeraries, and that crustaceans should be added to the already long list of organisms in which such chromosomes have been found (see White, 1954, p. 167). The genetic significance of supernumerary chromosomes remains to a great extent undecided, and light might well be thrown upon this problem by further work on the easily reared and cytologically manageable *G. pulex*.

SUMMARY

The chromosomes of nine species of gammarids have been studied in oocytes and embryos. There is no evidence that polyploidy has played a part in gammarid evolution. Three of the species which we have studied, *Gammarus pulex* (L.), *Marinogammarus marinus* (Leach) and *M. pirloti* (Sexton & Spooner) show chromosomal polymorphism. There is no evidence that this polymorphism depends on the existence of a Robertsonian 'fragmentation-fusion' system; rather it appears to be due to the presence of supernumerary chromosomes.

REFERENCES

HUXLEY, J. S., 1923. Further data on linkage in *Gammarus chevreuxi*, and its relation to cytology. *J. exp. Biol.*, Vol. 1, pp. 79–96.

LE CALVEZ, J., 1949. Quelques nouveaux caryotypes de Crustacés Amphipodes. *C.R. Acad. Sci., Paris*, T. 228, pp. 427–428.

LE CALVEZ, J. & CERTAIN, P., 1951. *Gammarus chevreuxi* Sext. et la caryologie des Gammariens. *Arch. Zool. exp. gén.*, T. 88, pp. 131–141.

LE ROUX, M. L., 1933. Recherches sur la sexualité des Gammariens. *Bull. Biol.*, Suppl., Vol. 16, 138 pp..

NIIYAMA, H., 1935. The chromosomes of *Paralithodes camtschatica* and *Gammarus annandalei*. *Jap. J. Genet.*, Vol. 11, pp. 34–35.

PALMER, R., 1926. The chromosome complex of *Gammarus chevreuxi* Sexton. I. Spermatogenesis. *Quart. J. micr. Sci.*, Vol. 70, pp. 541–551.

POISSON, R. & LE CALVEZ, J., 1948. La garniture chromosomique de quelques Crustacés Amphipodes. *C.R. Acad. Sci., Paris*, T. 227, pp. 228–230.

ROBERTSON, W. R. B., 1916. Chromosome studies. I. Taxonomic relationships shown in the chromosomes of Tettigidae and Acrididae. V-shaped chromosomes and their significance in Acrididae, Locustidae and Gryllidae: chromosomes and variation. *J. Morph.*, Vol. 27, pp. 179–331.

STAIGER, H., 1954. Der Chromosomendimorphismus beim Prosobranchier *Purpura lapillus* in Beziehung zur Ökologie der Art. *Chromosoma*, Bd. 6, pp. 419–478.

STAIGER, H. & BOCQUET, CH., 1954. Cytological demonstration of female heterogamety in isopods. *Experientia*, T. 10, pp. 64–66.

WHITE, M. J. D., 1954. *Animal Cytology and Evolution*. Cambridge University Press.

EXPLANATION OF PLATE I

Photomicrographs of 'squash' preparations stained in aceto-orcein: all figures at the same magnification.

Fig. 1. Polar view of first meiotic metaphase in spermatocyte of *Gammarus pulex*.

Fig. 2. Side view of first meiotic metaphase in oocyte of *G. pulex*: 26 bivalents.

Fig. 3. Side view of second meiotic metaphase in oocyte of *G. pulex*: 26 chromosomes.

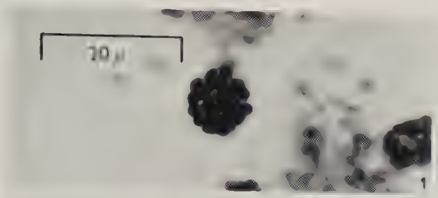
Fig. 4. Side view of second meiotic metaphase in oocyte of *G. pulex*: 27 chromosomes.

Fig. 5. Prometaphase of first mitotic division in fertilized egg of *G. pulex*: 52 chromosomes.

Fig. 6. Side view of second meiotic metaphase in oocyte of *Marinogammarus obtusatus*: 26 chromosomes.

Fig. 7. Side view of second meiotic metaphase in oocyte of *M. pirloti*: 29 chromosomes.

Fig. 8. Side view of second meiotic metaphase in oocyte of *M. pirloti*: 32 chromosomes, of which the one on the extreme right is the product of the equational division of a univalent during first meiosis.



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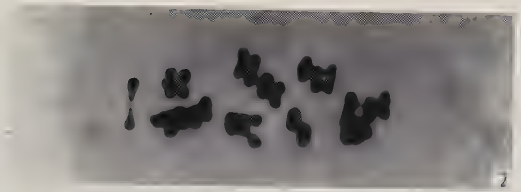

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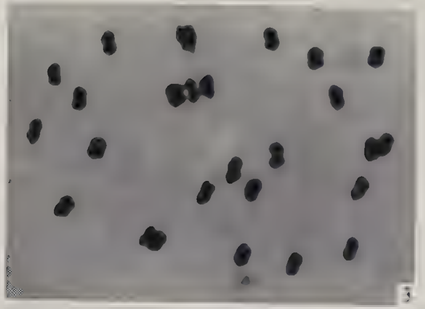

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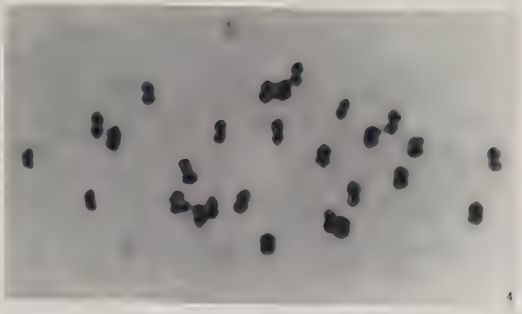

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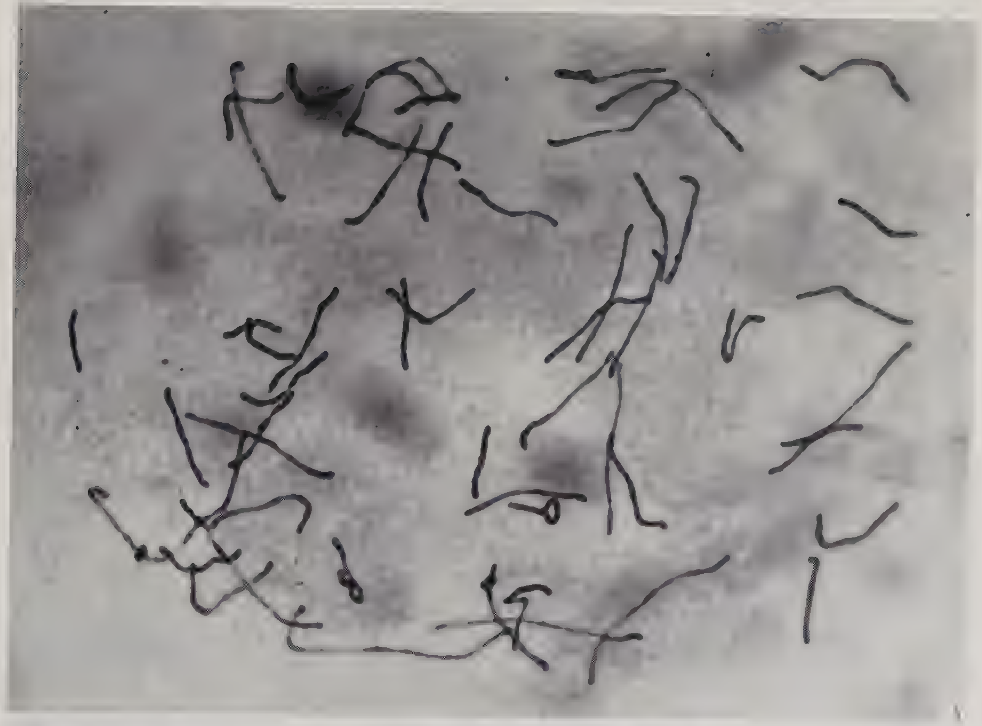

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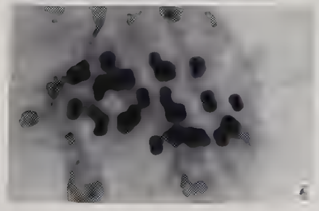

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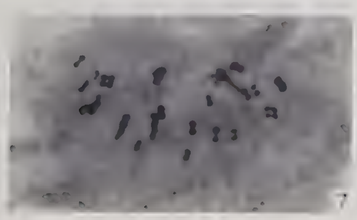

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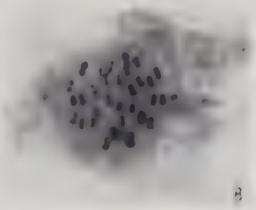

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SEASONAL VARIATIONS IN HOST-PARASITE RELATIONS BETWEEN FISH AND THEIR PROTOZOA

By Elmer R. Noble

University of California, Santa Barbara College

(Text-fig. 1)

Most papers on the subject of parasitology have been confined to descriptions and lists of parasites and to clinical and economical considerations. Ecological studies are more subject to errors of interpretation, and they usually require more time to complete. For these reasons an ecological approach to the study of parasitology has not been popular, but through such an approach the parasitologist has an opportunity to extend the range of his contributions to an understanding of basic problems in biology.

The term 'parasite' generally implies injury to a host, but parasitologists usually assume the absence of injury when visible evidence of it is lacking. Physiological injury may well exist in a healthy-looking animal. Parasites such as *Entamoeba histolytica* are injurious only occasionally, and are normally commensal in their relationship to the host. For these reasons I shall employ the term 'parasite' in a broad sense to include all organisms which normally nourish themselves within or on the body of another organism without destroying the latter.

Only one year was at my disposal for the present study, but I felt that a regular and systematic examination of the Protozoa and their fish hosts during the seasons of that year should result in information of value. I wish to emphasize that this report is presented primarily to suggest an ecological approach, often neglected, to problems in parasitology. Any significant conclusions on seasonal variations should be based upon at least three full annual cycles. My results, therefore, can only serve as a basis for further studies. The work was done at the Laboratory of the Marine Biological Association of the United Kingdom, Plymouth, from 28 July 1955 to 1 June 1956. I am exceedingly grateful to the Director of the Laboratory, Mr F. S. Russell, and his staff, who generously placed all necessary equipment and supplies at my disposal. Dr C. E. Lucas of the Marine Laboratory, Aberdeen, and Mr E. Ford of the Marine Station, Millport, were very helpful in supplying material from Scottish waters. I wish also to acknowledge receipt, from the National Science Foundation, Washington, of a research grant which made my trip to England possible.

METHODS

My studies were restricted to the Protozoa because an adequate attention to these forms, especially to the living parasites, consumed all of the available time. The fish used were the Lemon Sole (*Microstomus kitt*), the Dragonette (*Callionymus lyra*) and the Whiting (*Gadus merlangus*). They were collected by means of an otter trawl within about 10 miles of the port of Plymouth. The fish were brought immediately to the laboratory, and were alive when I started work on them, or they were so recently dead that the blood flowed freely when the heart was cut. If the blood did not flow freely the fish was discarded. Blood for smears was obtained from the heart immediately after incision by a razor blade. In *M. kitt* the incision was made anterior to the liver after the peritoneal cavity was opened; in *G. merlangus* it was made between the gills before the body was opened; and in *C. lyra* it was made ventrally through the body wall. Before each incision the area to be cut was wiped with absorbent paper in order to reduce the possibility of contamination.

Macroscopic examinations of the following organs of each fish were made: skin, fins, mouth, pharynx, gills, viscera, kidney and peritoneal cavity. I recorded the appearance of all unusually heavy infections of worms, and gill infections by the copepod *Lernaea*. Other copepods were very seldom observed. Leeches were never found on the fish or in the water of the containers in which the fish were brought to the laboratory.

Microscopic examinations of the blood, gall bladder and gut contents were made for each fish. Occasionally, examinations of the stomach contents, urinary bladder and other organs were also made. The gut and gall bladder contents were examined in the fresh condition immediately after collection so that living parasites, if present, were easily observed. Relatively few permanent, stained preparations from these organs were prepared. Schaudinn's or Susa's fluids with iron haematoxylin were usually employed. The blood smears were fixed for from 5 to 10 min in methyl alcohol as soon as they were dry, then stained for from 30 to 60 min in Giemsa diluted with a phosphate buffer solution.

Abundant evidence from numerous workers indicates that although young fish may have a high percentage of parasitic infection, the intensity is low. Adult fish have the most parasites because of their intensive feeding habits during growing stages, including the feeding on intermediate hosts, and because of an accumulation of parasites (see Dubinin, 1936). For these reasons I generally selected the larger fish for study. No particular effort was made to separate males from females, but no differences in the degree or kind of infection between the sexes were noted. A maximum of fifteen fish (usually five of each species) was studied at one time because I found that more than this number required so much time to dissect and examine, that degeneration of host cells and death of the parasites (especially flagellates) reduced the chances of their detection in fresh preparations.

THE HOSTS

Of the fish studied, *Microstomus kitt* and *Callionymus lyra* are bottom feeders, and both are to be found in the same localities. If parasites of one have a wide range of host tolerance, the other fish might be expected to be host to the same parasites. *M. kitt* feeds primarily on polychaete worms which are the predominant fauna in the mud frequented by the fish. *C. lyra*, on the other hand, feeds upon practically any bottom-dwelling animal of suitable size; e.g. small crustaceans, annelids, arthropods and molluscs. It has been well established that differences in feeding habits are related to differences in parasites, especially when intermediate hosts are involved.

The two species of fish mentioned above are widely separated taxonomically. *Microstomus kitt* is a flatfish belonging to the family Pleuronectidae. *Callionymus lyra* is a member of the family Callionymidae, and is of a more conventional fish shape, except that its mouth is ventrally placed. It is brilliantly coloured, and the sexes are strikingly different in their external features. The third species of fish, *Gadus merlangus*, belongs to the family Gadidae. It is an active predator, feeding chiefly upon smaller fish, with a greater vertical range of habitat than is characteristic of the other two species.

Dr G. A. Steven of the Plymouth Marine Laboratory has been keeping records of the numbers of fish of each species taken by otter trawl during the past several years at Plymouth. Although the records are incomplete, the chart shows that *Callionymus lyra* tends to be found in greater numbers during the warmer months, but that the other two species of fish do not fluctuate in any consistent manner throughout the year. The records are tabulated in Table 1.

TABLE 1. NUMBER OF FISH PER HAUL OF ONE HOUR AT PLYMOUTH, ENGLAND

Figures show an average usually of three or four hauls. From the records of Dr G. A. Steven (unpublished).

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
Callionymus lyra	201	161	185	169	151	370	335	572	373	548	419	241	1953
Gadus merlangus	>1	1	28	87	45	204	286	67	5	2	0	0	
Microstomus kitt	9	7	10	8	5	7	3	6	8	3	0	6	
C. lyra	239	4	42	236	N.R.*	N.R.	N.R.	N.R.	200	385	611	176	1954
G. merlangus	>1	0	7	33	N.R.	N.R.	N.R.	N.R.	9	37	0	11	
M. kitt	4	10	11	9	N.R.	N.R.	N.R.	N.R.	6	2	4	8	
C. lyra	94	91	98	79	168	437	377	294	N.R.	57	82	105	1955
G. merlangus	20	63	85	168	27	20	7	13	N.R.	5	1	0	
M. kitt	13	7	6	21	9	13	7	9	N.R.	1	2	0	
C. lyra	136	N.R.	N.R.	243	192	545	—	—	—	—	—	—	1956
G. merlangus	7	N.R.	N.R.	29	8	27	—	—	—	—	—	—	
M. kitt	4	N.R.	N.R.	15	4	11	—	—	—	—	—	—	

* N.R. = no record.

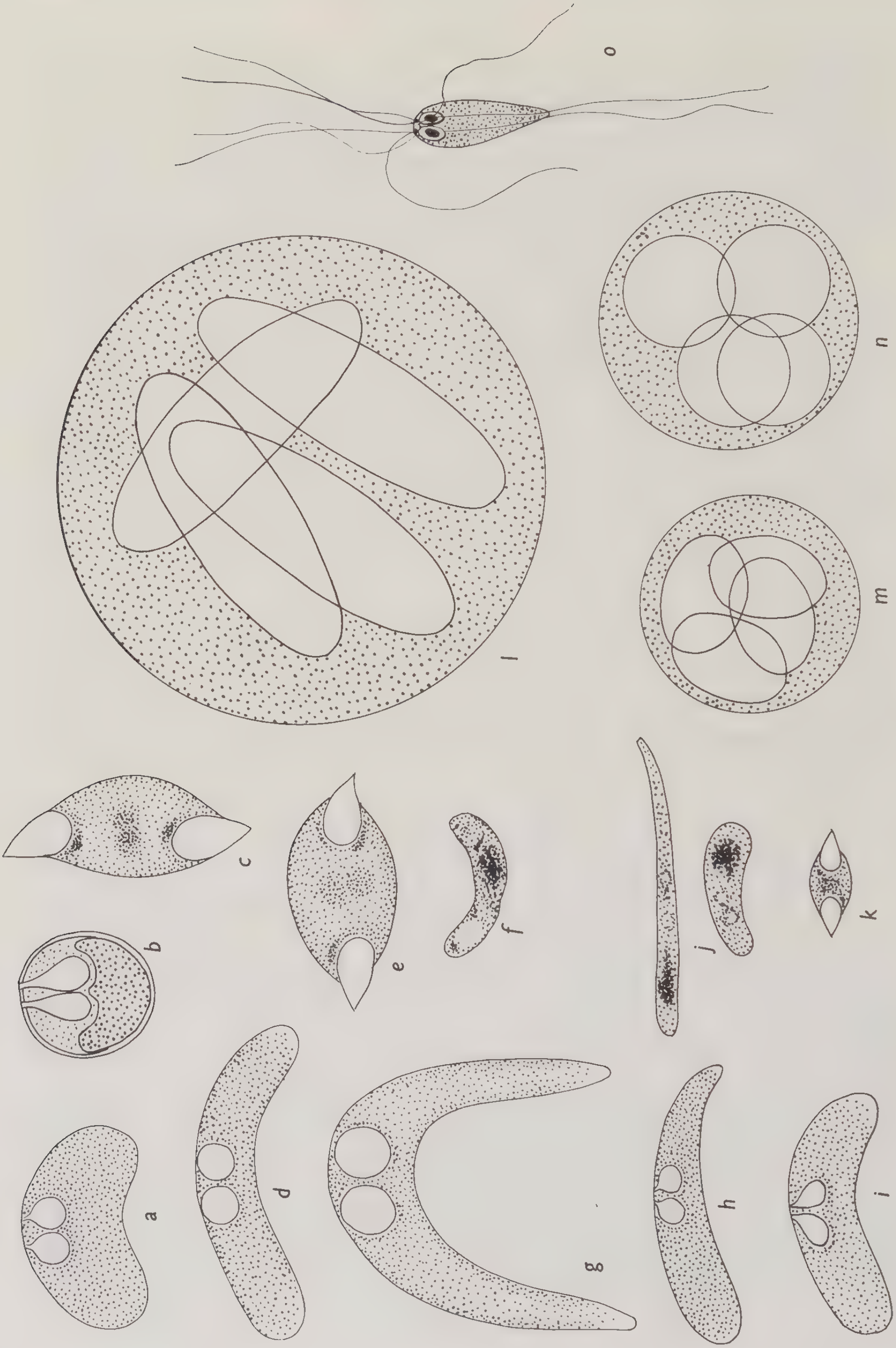
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Fig. 1

THE PROTOZOAN FAUNA

It is distressing to find statements in the literature indicating that a new species name has been created solely or even primarily because the parasite is found in a new species of host. Such a practice is entirely unwarranted. It is well known that many species of parasites have a wide range of host tolerance, and unless there are obviously-marked differences in morphology, and unless the entire life cycle is known, and sufficient numbers of individuals have been observed and measured, the description of a new species is unjustified. Too little attention has been paid to physiological differences which should be as important as morphological ones in the distinctions among species. Physiological characteristics are, of course, more difficult to identify, but this fact should not result in their being ignored. For these reasons I shall not attempt, at this time, descriptions or species designations of the forms which appear to be new. The figures illustrated in Fig. 1 are semi-diagrammatic to show only general form and relative sizes of the Protozoa which I have found. Lists of the Protozoa for each of the three fish are shown in Tables 2–4.

I have seen many forms intermediate in appearance between the two species of *Callionymus*-infecting haemogregarines described by Brumpt & Lebailly, and I believe that only one species is present. Reichenow (1932) suggested that *Haemogregarina callionymi* is the sexual form of *H. quadrigemina*. *H. binucleata* was mentioned in 1910 by Henry but he did not describe it. It also is probably *H. quadrigemina*. The two unspecified *Ceratomyxa*, one from the gall bladder, the other from the urinary bladder, and the *Eimeria* may be identical with species reported from other hosts, but, as indicated above, I shall not attempt a description of them at this time. *Eimeria* in the liver of *Callionymus* was frequently abundant enough to give it a speckled-white appearance. In such heavy infections the oocysts were usually found in large numbers in the gall bladder.

As regards the whiting, Auerbach (1910) distinguished *Myxidium bergense* from *M. sphaericum* Thélohan chiefly on the basis of the trophic stages which

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Legend to Fig. 1

Fig. 1. Semi-diagrammatic drawings of Protozoa obtained from fish, intended only to show general outlines and relative size. Scale uniform. *a*, *Leptotheca informis* from gall bladder of *Gadus merlangus*. *b*, *Myxobolus aeglefini* from eye cartilage of *Gadus merlangus*. *c*, *Myxidium sphaericum* from gall bladder of *Gadus merlangus*. *d*, *Ceratomyxa lata* from gall bladder of *Microstomus kitt*. *e*, *Myxidium* sp. from gall bladder of *Microstomus kitt*. *f*, *Haemogregarina platessae* from blood of *Microstomus kitt*. *g*, *Ceratomyxa* sp. from urinary bladder of *Callionymus lyra*. *h*, *Ceratomyxa* sp. from gall bladder of *Callionymus lyra*. *i*, *Ceratomyxa arcuata* from gall bladder of *Callionymus lyra* and *Gadus merlangus*. *j*, *Haemogregarina quadrigemina* from blood of *Callionymus lyra*. *k*, *Myxidium incurvatum* from gall bladder of *Callionymus lyra* and *Gadus merlangus*. *l*, *Eimeria* sp. from gut of *Gadus merlangus*. *m*, *Eimeria* sp. from gut of *Gadus merlangus*. *n*, *Eimeria* sp. from liver of *Callionymus lyra*. *o*, *Hexamita* sp. from stomach and gut of *Gadus merlangus*.

in the former species has medium-pointed pseudopodia and a length up to 54μ, while in the latter it is rounded, about 20μ in diameter with lobed pseudopodia. My observations suggest that these are the same species because there is a great deal of variation in dimensions and appearance of the trophic stages, depending on temperature, freshness of the preparation, age of parasites, etc. I could find no evidence for two distinct kinds of *Myxidium* spores within the size ranges of *M. bergense* and *M. sphaericum*.

TABLE 2. PROTOZOA IN *CALLIONYMUS LYRA*

Previously reported	Found during present study	Percentage of hosts infected
Blood		
Haemogregarina quadrigemina Brumpt & Lebailly, 1904 (Europe) Report from Plymouth by Henry, 1913	*H. quadrigemina* (Fig. 1*j*)	70
H. callionymi Brumpt & Lebailly, 1904 (Europe) Report from Plymouth by Henry, 1910	—	—
H. binucleata Henry, 1910, from Plymouth	—	—
Trypanosoma callionymi Brumpt & Lebailly, 1904 (Europe) Report from Plymouth by Henry, 1913	—	—
Gall bladder		
Ceratomyxa arcuata Thélohan, 1892 (Europe) Report from Plymouth by Dunkerley, 1920	*C. arcuata* (Fig. 1*i*) *C.* sp. (Fig. 1*h*)	62
Myxidium incurvatum Thélohan, 1892 Report from Plymouth by Dunkerley, 1920	*M. incurvatum* (Fig. 1*k*)	92
Liver		
—	*Eimeria* sp. (Fig. 1*n*)	9
Urinary bladder		
—	*Ceratomyxa* sp. (Fig. 1*g*)	(4 out of 8)
Muscle		
Chloromyxum quadratum Thélohan, 1894 (Europe)	—	—
Nosema destruens (Thélohan, 1891), Labbé, 1899 (Europe)	—	—

The *Eimeria* of the gut of *Gadus merlangus* undoubtedly belong to two species because of the marked differences in shape and size of oocysts. *Hexamita* in the stomach and gut is very similar to several species which have been described from unrelated hosts (see Wenrich, 1935). This genus is remarkable in its lack of host specificity, and I hope to make a special study of the form in *Gadus merlangus*, and relate it to the more general problem of host-specificity and origin of parasitism. *Myxobolus aeglefini* was found in only one out of the 200 fish examined.

TABLE 3. PROTOZOA IN *GADUS MERLANGUS*

Previously reported	Found during present study	Percentage of hosts infected
Gall bladder		
Ceratomyxa arcuata Thélohan, 1892 (Europe) Report from Plymouth by Dunkerley, 1920	*C. arcuata* (Fig. 1*i*)	2
Leptotheca informis Auerbach, 1910 (Europe)	*L. informis* (Fig. 1*a*)	17
Myxidium bergense Auerbach, 1910 (Europe)	*M. incurvatum* (Fig. 1*k*) Thélohan, 1892	3
M. sphaericum Thélohan, 1894 (Europe) Report from Plymouth by Dunkerley, 1920	*M. sphaericum* (Fig. 1*c*)	58
Gills		
Amyloodinium ocellatum (Brown), 1934 (England)	—	—
Bone and cartilage		
Myxobolus aeglefini Auerbach, 1906 (Europe)	*M. aeglefini* (Fig. 1*b*)	0·5
Pyloric caecae		
Mrázesia piscicola Cépède, 1924 (Europe)	—	—
Stomach and intestine		
—	*Eimeria* sp. no. 1 in gut (Fig. 1*l*)	0·5
—	*Eimeria* sp. no. 2 in gut (Fig. 1*m*)	1·5
—	*Hexamita* sp. in gut and stomach (Fig. 1*o*)	4

TABLE 4. PROTOZOA IN *MICROSTOMUS KITT*

Previously reported	Found during present study	Percentage of hosts infected
Blood		
Haemogregarina platessae Lebailly, 1904 (Europe) Report from Plymouth by Henry, 1913	*H. platessae* (Fig. 1*f*)	1·1
H. flesi Lebailly, 1904 (Europe)	—	—
Gall bladder		
Ceratomyxa awerinzewi Reichenow, 1929 (Europe)	*Myxidium* sp.	11
Ceratomyxa lata Dunkerley, 1920 (Plymouth)	*C. lata* (Fig. 1*d*)	97

The *Myxidium* from *Microstomus kitt* appears very similar to *M. bergense* Auerbach originally described from *Gadus virens*. *Ceratomyxa lata* seems to have two forms (possibly two species), one with relatively large (average 5·2 *μ* in diameter) polar capsules, and the other with smaller (average 3·2 *μ*) polar capsules. The size of the spore (9 by 25·7 *μ*) is somewhat larger than that originally reported by Dunkerley. *Haemogregarina platessae* and *H. flesi* seem to be identical (see Reichenow, 1932). Ten schizonts from my preparations of blood smears averaged 2·4 by 8·4 μ.

SEASONAL VARIATIONS

The intensity of infection was estimated on the basis of relative numbers of individual parasites as seen through the microscope, using an oil-immersion objective and 10× ocular. One to about fifteen parasites in a field was designated as +. If the field was crowded with parasites it was designated as + + +, and everything between these two was listed as + +. The obvious limitations of this method did not prevent a reasonably accurate indication of shifts of infection intensity, but for definite and convincing results a more precise method would have to be used, and the results subjected to a statistical analysis. I arbitrarily divided the year into three periods roughly corresponding to summer, winter and spring. The times of my arrival in and departure from Plymouth prevented collections of fishes during the month of June and most of July. The results are listed in Tables 5–7.

Material from a small number of the same three species of fish collected at Millport and at Aberdeen during the month of October 1955 indicated similar degrees of infection as those found at Plymouth during the same month.

Callionymus lyra (Table 5)

Some evidence for seasonal changes is to be found in this fish. Heavy infections of myxosporidia in the gall bladder occurred in 19% of the summer fish, and rose to 40% in winter fish. At the same time, however, and in the same fish, haemogregarines of the blood showed a reverse tendency. The blood in 24% of the fish was heavily infected during the summer, while only 7% were heavily infected during the winter.

TABLE 5. *CALLIONYMUS LYRA*, SEASONAL DATA

		Percentage of fish infected			
	Intensity of infection	Summer 28 July to 31 Oct. 76 fish	Winter 1 Nov. to 29 Feb. 62 fish	Spring 1 Mar. to 26 May 62 fish	Total of 200 fish
Myxosporidia in gall bladder	–	0	2	0	0·7
	+	39	14	27	27
	+ +	42	44	46	44
	+ + +	19	40	27	28·3
Haemogregarines in blood	–	36	28	35	33
	+	40	66	48	51
	+ +	24	7	17	16

Twice as many spring fish were infected with gill copepods (*Lernaea*) and internal worms as were the summer and winter fish. Liver infection with *Eimeria* occurred in one summer fish, four winter fish and thirteen spring fish. Worm infections were never heavy and no general correlations between the intensities of Protozoa and worms were noted in *Callionymus lyra*. The gall bladders of 99·3 % of the fish contained myxosporidia, and 66 % of these fish were infected with haemogregarines.

Microstomus kitt (Table 6)

There was a slight increase in numbers of winter fish with heavy gall bladder infections, but the small numbers involved preclude any generalization. Cysts of larval worms were occasionally found in the viscera. Of the total fish, over 99 % were infected with myxosporidia.

TABLE 6. *MICROSTOMUS KITT*, SEASONAL DATA

		Percentage of fish infected			
	Intensity of infection	Summer 28 July to 31 Oct. 70 fish	Winter 1 Nov. to 29 Feb. 51 fish	Spring 1 Mar. to 26 May 55 fish	Total of 176 fish
Myxosporidia in gall bladder	−	3	0	0	1
	+	93	53	62	69
	++	4	33	33	24
	+++	0	14	5	6

Gadus merlangus (Table 7)

No seasonal changes are indicated. Heavy worm infections (predominantly nematodes) appeared to be more abundant during the spring period. Of the total fish, 79 % were infected with myxosporidia.

TABLE 7. *GADUS MERLANGUS*, SEASONAL DATA

		Percentage of fish infected			
	Intensity of of infection	Summer 28 July to 31 Oct. 61 fish	Winter 1 Nov. to 29 Feb. 74 fish	Spring 1 Mar to 26 May 65 fish	Total of 200 fish
Myxosporidia in gall bladder	−	23	16	26	21
	+	45	57	56	53
	++	23	19	12	18
	+++	9	8	6	8

PATHOLOGY

Protozoan parasites, in general, appear to be of a commensal nature in fishes, but very little work has been done on the effects of parasites on the growth and development of these hosts. The most commonly infected organ in all the fish examined was the gall bladder, containing myxosporidian parasites. Infected bile undergoes chemical changes as indicated by a yellow colour and changes in density and viscosity. In a heavily infected gall bladder the bile often becomes so viscous that it does not flow when the shrunken bladder is cut open. Its contents are frequently of a white, cheese-like consistency. Similar pathological changes, however, may appear when there is no visible evidence of the presence of myxosporidia or of bacteria, and the pathological changes are presumably due, in these instances, to a virus infection.

The only other infected organs found to exhibit visible signs of pathology were the eyes of one specimen of *Gadus merlangus*, the cornea and sclera of which were spotted by opaque, white cysts of the myxosporidian, *Myxobolus aeglefini*; and the liver of *Callionymus lyra* which was occasionally speckled with cysts of *Eimeria*. One observation not indicated in the previous tabulations of results, and often noted by parasitologists, was the apparent deleterious effect on one species of parasite by the presence of unusual numbers of another species of parasite in the same host body. For example, during massive infections of nematode worms, Protozoa were greatly reduced in numbers, or they were absent.

DISCUSSION

If one may assume that myxosporidia are consistently abundant in the gall bladders of *Callionymus lyra* in summer, and that haemogregarines are consistently more abundant in the blood in winter (an assumption based on little evidence), one might correlate these changes with population fluctuations of the hosts (see Table 1). One might assume that the Plymouth area during the summer months is more favourable for intermediate hosts of haemogregarines and less favourable for infection with myxosporidian spores than during the winter months. But we do not know whether the fish that are caught during the summer months are those that are left behind when most of the population migrates to places unknown, or whether they represent fish which have moved into the Plymouth area during the summer season from elsewhere. We are not even sure of the identity of the intermediate hosts.

Nothing is known, as far as I am aware, of the migratory habits of the fish used in this study. Are the bottom-dwellers more restricted in their range than is *Gadus merlangus*? *Callionymus lyra* generally has blood Protozoa as well as gall bladder Protozoa, and occasionally also copepods on its gills. *Microstomus kitt* rarely has blood parasites, and I found no gill copepods. Are these differences correlated with differences in habitats? A basis for some hypotheses about their migratory habits might be found among conclusions drawn by other workers who have been interested in similar problems dealing with freshwater fish. Bychowskaja (1936), for example, found that fish in small lakes have fewer numbers and kinds of parasites than do fish in large lakes. It occurred to me that perhaps marine fish in a restricted habitat have fewer parasites than marine fish which roam over a wide area. Unfortunately, few studies have been made on migrations of marine fishes, and, while bottom-dwelling fish might be expected to be the more restricted in their range, I know of no proof for the assumption. One might predict, however, on the basis of their respective parasitic faunas, that *C. lyra* has a wider migratory range than has *M. kitt*. Gorbunova (1936) studied the variations of the parasites of freshwater pike and roach with respect to their ages. She found that, in addition to an increase in numbers of parasitic species with the ages of the hosts, the fauna in fish of quieter habits is poorer than that in the predatory fish (i.e. pike). Again applying these findings to my own studies, one could correlate the relatively poorer protozoan fauna exhibited by *M. kitt* with the presumably more restricted and 'quieter' bottom habitat which the host occupies.

Gadus merlangus has a diet restricted chiefly to small fish, but there appeared to be more varieties of fish in its stomach than varieties of annelids in the stomach of *Microstomus kitt*. In addition, *G. merlangus* probably has a wider migratory range than has *M. kitt*. These factors would suggest a higher degree of parasitism in *G. merlangus*, and my records show that ten species of Protozoa have been reported from this fish as compared with four species from *M. kitt*. In addition, *G. merlangus* is much more heavily infected with worms than is either *Callionymus lyra* or *M. kitt*. *C. lyra*, on the other hand, consumes a wide variety of food, and it has demonstrated a marked seasonal fluctuation in population numbers. It has about the same numbers of kinds of protozoan parasites as has *G. merlangus*, but the percentage of hosts infected with Protozoa is considerably higher.

It is surprising that my search for blood parasites in 200 stained slides, each from a different *Callionymus lyra*, did not reveal a single trypanosome. The fact that in 1910 Henry found, at Plymouth, six out of fifteen of these fish to be infected with trypanosomes suggests that they are subject to a yearly fluctuation of trypanosome infection. The fluctuation may be due to a periodic disappearance of leeches which serve as intermediate hosts, but haemogregarines are also transmitted by leeches, and haemogregarines were common in *C. lyra*, and occasionally they were found in *Microstomus kitt*. In fact, the abundance of haemogregarines in *C. lyra*, with the absence of leeches, throws doubt on the assumption that these parasites in this fish are transmitted by leeches. A few copepods attached to the gills were examined for haemogregarines but the results were negative.

The results of my studies, together with those of other writers, suggest the following tentative conclusions. An active predatory habit predisposes to heavy parasitism, but this result may be off-set by a restriction of the diet to one or to a few kinds of food species. Likewise, a less active host may acquire numerous kinds of parasites because of a taste for a wide variety of foods. A sluggish bottom-feeder with little tendency toward migration, and with limited tastes for food variety, tends to acquire relatively few kinds and numbers of parasites.

I have raised more questions than I have answered, but these questions suggest several lines of research which should be of significance in the field of ecological parasitology. Mere descriptions of parasites and their life cycles (including those which I have previously authored) generally carry with them implications of completeness which can be grossly misleading. Such descriptions fail to impress the reader with the impact of environmental changes, both internal and external, upon host-parasite relationships.

SUMMARY

From July 1955 to June 1956 a study of host-parasite relations between three species of marine fish and their Protozoa was made at Plymouth. *Callionymus lyra*, a bottom-dweller, tends to be more abundant during the summer months, consumes a wide variety of food, and was more heavily infected with Protozoa than were the other two species of fish. *Gadus merlangus* is an active predator, feeding on small fish, and although it has about the same numbers of kinds of Protozoa as does *C. lyra*, it was less heavily parasitized. *G. merlangus*, however, was more heavily parasitized with worms than were the other two fish. *Microstomus kitt* is a sluggish bottom-dweller, feeding on annelids, and, although over 99% were infected with myxosporidia, the intensity of infection was generally low. Very few other parasites were found in *M. kitt*.

An active predatory habit combined with a taste for a wide variety of food appears to predispose to heavy parasitism. Some evidence for seasonal variations in intensities of protozoan infection in *Callionymus lyra* was obtained, but for conclusive results more precise methods of measuring numbers of parasites, and further studies carried on over a period of at least three consecutive years, must be made. The report is presented primarily to emphasize the importance of an ecological approach to the study of parasitology.

REFERENCES

Auerbach, M., 1906. Ein Myxobolus im Kopf von *Gadus aeglefinus* L. *Zool. Anz.*, Bd. 30, pp. 568–70.

—— 1910. Biologische und morphologische Bemerkungen über Myxosporidien. *Zool. Anz.*, Bd. 35, pp. 57–63.

Brown, Eleanor M., 1934. On *Oodinium ocellatum* Brown, a parasitic dinoflagellate causing epidemic diseases in marine fish. *Proc. zool. Soc. Lond.*, pp. 583–607.

Brumpt, E. & Lebailly, C., 1904. Description de quelques nouvelles espèces de Trypanosomes et d'Hémogrégarines parasites des Téléostéens marins. *C.R. Acad. Sci., Paris*, T. 139, pp. 613–15.

Bychowskaja, I., 1936. The influence of the dimensions of the waterbasins on the parasitic fauna of fish. In V. A. Dogiel's 'Problems of ecological parasitology'. *Ann. Leningrad State Univ.*, No. 7, pp. 163–6. (In Russian.)

Cépède, C., 1924. *Mrázekia piscicola* n.sp. microsporidie parasite du merlan *Gadus merlangus* Linné. *Bull. Soc. zool. Fr.*, T. 49, pp. 109–13.

Dubinin, V. B., 1936. Investigation of the parasitic fauna of *Thymallus vulgaris* in various periods of its life. In V. A. Dogiel's 'Problems of ecological parasitology'. *Ann. Leningrad State Univ.*, No. 7, pp. 31–48. (In Russian.)

Dunkerley, J. S., 1920. Fish Myxosporidia from Plymouth. *Parasitology*, Vol. 12, pp. 328–33.

GORBUNOVA, M., 1936. Variations of the parasitic fauna of the pike and the roach with their age. In V. A. Dogiel's 'Problems of ecological parasitology'. *Ann. Leningrad State Univ.*, No. 7, pp. 5–30. (In Russian.)

HENRY, H., 1910. On the haemoprotozoa of British sea-fish (a preliminary note). *J. Path. Bact.*, Vol. 14, pp. 463–5.

HENRY, H., 1913. A list of blood parasites of sea-fish taken at Plymouth. *J. mar. biol. Ass. U.K.*, Vol. 9, pp. 570–1.

LABBÉ, A., 1899. Sporozoa. *Das Tierreich*, Bd. 5, 180 pp.

LEBAILLY, C., 1904. Sur quelques hémoflagellés des téléostéens marins. *C.R. Acad. Sci. Paris*, T. 139, pp. 576–7.

REICHENOW, E., 1929. Sporozoa, Ciliata und Suctoria. In Doflein, F. & Reichenow, E., *Lehrbuch der Protozoenkunde* (Aufl. 5), Teil 2.

—— 1932. Sporozoa. *Tierwelt N.- u. Ostsee*, Teil 2, g, pp. 1–88 (Lief. 21).

THÉLOHAN, P., 1891. Sur deux sporozoaires nouveaux, parasites des muscles des poissons. *C.R. Acad. Sci. Paris*, T. 112, pp. 168–71.

—— 1892. Sur quelques coccidies nouvelles parasites des poissons. *J. Anat., Paris*, T. 28, pp. 151–71.

—— 1894. Recherches sur les myxosporidies. *Bull. sci. Fr. Belg.*, T. 26, pp. 100–394.

WENRICH, D. H., 1935. Host-parasite relations between parasitic Protozoa and their hosts. *Proc. Amer. phil. Soc.*, Vol. 75, pp. 605–50.

J. mar. biol. Ass. U.K. (1957) 36, 157–160
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BIO-ASSAY OF NITROGEN AVAILABLE TO TWO SPECIES OF PHYTOPLANKTON IN AN OFF-SHORE WATER

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In a series of preliminary trials, off-shore water, collected during the summer of 1955 and containing only small concentrations of nitrogen and phosphorus available to plants, was filtered, pasteurized, enriched with phosphate, manganese and iron, inseminated with washed *Phaeodactylum tricornutum* and portioned into a series of flasks. To these were added varying quantities of nitrate, raising the concentration, in steps, up to 40 μg nitrate-N per litre. The flasks were illuminated, either in a north window or in front of a 'warm daylight' fluorescent strip, precautions being taken to prevent contamination with ammonia or oxides of nitrogen in the atmosphere. Growth of the algae was followed by withdrawing samples at intervals, after shaking, and measuring their optical density in a 10 cm cuvette.

After an initial lag period and the following period of exponential growth, during which the nitrogen source was used and consequently synthesis of chlorophyll ceased, the rate of increase in optical density slowed, and persisted at a regular slow rate for several days.

During this post-exponential period of growth the increase in optical density due to added nitrate was almost directly proportional to the quantity of nitrogen source which had been added.

The observations of optical density (light path 10 cm), shown in Table 1, were made in an experiment after 7, 9 and 11 days illumination. The initial O.D. (10 cm) of the inseminated water was 0·007, and the inseminum was nitrogen-deficient and had a long lag period before growth started.

TABLE 1. INCREASE IN OPTICAL DENSITY DURING POST-EXPONENTIAL PERIOD

Flask no.	O.D. (10 cm) after ...	7 days	9 days	11 days
1	With no added nitrate	0·035	0·033	0·050
2		0·041	0·046	0·050
3	+ 20 μg/l. NO_3N	0·063	0·070	0·078
4		0·066	0·071	0·080
5	+ 40 μg/l. NO_3N	0·084	0·101	0·106
6		0·085	0·102	0·114
7	—	0·090	0·095	0·112
Increase due to addition:				
of 20 μg N/l.		0·026	0·030	0·029
of 40 μg N/l.		0·049	0·059	0·060

In order to assay the available nitrogen present in a sample of water the following reasoning was employed. Provided the washed plant cells added as inseminum were wholly deficient in nitrogen, and consequently incapable of further growth unless supplied with a nitrogen source, then, at any particular time T during post-exponential growth, the increase in optical density since the start of the experiment depends upon the concentration C of available nitrogen in the water, and equals Cy μg/N/l. if y is the increase in optical density due to the addition of 1 μg nitrate-N/l.

If, however, the plant cells added as inseminum were growing actively and capable of further carbon synthesis without a source of nitrogen, then the increase in optical density equals $Cy + x$. The value of x is directly proportional to the quantity of inseminum added. Therefore, x is equal to the difference in the increases in optical density which have taken place in samples to which one unit and two unit quantities of inseminum have been added.

Hence from observations of these two increases and of the increase due to a known addition of available nitrogen, the value of C can be calculated. Experiments indicated that throughout several days in which post-exponential growth was taking place, the value of C remained the same within the limits of experimental error.

A bio-assay was made in water collected 20 miles off shore from Plymouth on 13 July 1955, from a depth of 15 m.

The water was filtered, pasteurized, and enriched with 500 μg/l. of phosphate-P, 10 μg/l. of manganese and 10 μg/l. of iron as ferrous dipyridyl.

Samples of this enriched water were analysed by Dr J. P. Riley, and was found to contain 10 μg total inorganic nitrogen per litre by the method described by Riley and Sinhaseni (1957).

Part of the water was inseminated with *Phaeodactylum tricornutum*, the initial optical densities measured, and the flasks were kept in a north window. The optical densities were again measured on the fourth and sixth days, and from the observed increases on the sixth day the concentration C was calculated (Table 2).

On the fourth day the nitrate added to flasks 5–8 had not exerted its full effect upon carbon synthesis.

The remainder of the water was inseminated with *Chlorella stigmatophora* and treated in the same way, providing the observations shown in Table 3.

On the sixth day some of the cells had started to disintegrate; the post-exponential growth was of short duration compared with that of *Phaeodactylum*.

Since the average concentration of available nitrogen found by assay is similar to the concentration of total inorganic nitrogen found by analysis, it appears that only an insignificant quantity of organic nitrogen was used by these plants in the presence of their associated bacteria, although sea water contains a material quantity of organic nitrogen.

It is remarkable that so much organic nitrogen should remain in solution unattacked by bacteria in the sea—Krogh (1934) found some 240 μg organic-N/l. down to depths of 4750 m—whereas organic phosphorus is not found at depths below 1000 m.

TABLE 2. BIO-ASSAY OF CHANNEL WATER WITH *PHAEODACTYLUM*

		Optical density, 10 cm light path		
Flask no.		Initial	On 4th day	On 6th day
1	Unit inseminum	0·013	0·035	0·036
2		0·012	0·036	0·035
3		0·013	0·036	0·036
4		0·013	—	0·034
5	Unit inseminum + 40 μg NO_3N/l.	0·013	0·072	0·081
6		0·014	0·079	0·085
7		0·014	0·078	0·088
8		0·017	—	0·088
9	Double inseminum	0·022	0·052	0·053
10		0·023	0·052	0·054
11		0·027	0·055	0·057
12		0·026	—	0·057

Calculated concentration of available nitrogen, $C = 11{\cdot}5$ μg N/l.

TABLE 3. BIO-ASSAY OF CHANNEL WATER WITH *CHLORELLA*

		Optical density, 10 cm light path	
Flask no.		Initial	On 4th day
1	Single inseminum	0·011	0·066
2		—	0·061
3		—	0·066
4		—	0·063
5	Single inseminum + 40 μg NO_3N/l.	—	0·127
6		—	0·129
7		—	0·132
8		—	0·142
9	Double inseminum	0·025	0·107
10		—	0·127
11		—	0·100
12		—	0·107

Calculated concentration, C, of available nitrogen 13 μg/l.

SUMMARY

A bio-assay of the nitrogen which was available to two phytoplankton species with their associated bacteria in an off-shore water is described.

The concentrations found by assay (11·5 and 13 μg N/l.) were similar to the concentration of total inorganic nitrogen compounds found by analysis (10 μg N/l.).

Of the organic nitrogen normally present in solution in sea water, an insignificant quantity appears to be available to the plant-bacteria community.

REFERENCES

KROGH, A., 1934. Conditions of life at great depths in the ocean. *Ecol. Monogr.*, Vol. 4, pp. 430–39.

RILEY, J. P. and SINHASENI, P., 1957. The determination of ammonia and total ionic inorganic nitrogen in sea water. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 161–68.

J. mar. biol. Ass. U.K. (1957) 36, 161–168
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THE DETERMINATION OF AMMONIA AND TOTAL IONIC INORGANIC NITROGEN IN SEA WATER

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(Text-figs. 1 and 2)

Combined inorganic nitrogen occurs in sea water principally as nitrate, nitrite and ammonium ions, the concentrations of which lie in the ranges 1–600 mg NO_3^-–N/m^3, 0·1–50 mg NO_2^-–N/m^3 and 5–50 mg NH_4^+–N/m^3 respectively. Nitrogen occurring in any of these forms is readily assimilable by marine organisms, and its exhaustion in sea water is frequently a growth-limiting factor in the water. This paper describes a method for the determination of the total ionic inorganic nitrogen in sea water, based on preliminary reduction to ammonia followed by separation and estimation of the latter colorimetrically.

Riley (1953) has discussed the determination of ammonia in sea water, and has concluded that ammonia is best separated from the water, adjusted to pH 9·2, by distillation under reduced pressure in a current of air as described by Krogh (1934). The distillation process is rather time-consuming and requires a special apparatus. Experiments were therefore carried out to separate the ammonia by diffusion. It was found that the capacity of the concentric microdiffusion cells employed by Conway (1950, p. 8) was too small for the volume of sea water which was necessary if low concentrations of ammonia were to be determined. When diffusion was carried out in the flasks (Fig. 1) described by Cavett (1937) for the microdetermination of alcohol in blood, reproducible recoveries of approximately 73 % of added ammonium salt were obtained from 50 ml. of sea water after diffusion at 70° C for 24 h at pH 9·2 (Table 1).

Much of the earliest work on the determination of nitrate in sea water was based on its reduction to ammonia, which was separated by distillation and determined by Nessler's method. Few of the reducing agents (magnesium, Raben, 1905*a*, sodium amalgam, Raben, 1905*b*; aluminium amalgam, Raben, 1910, 1914; Devarda's alloy, Brandt, 1927; iron-zinc couple, Thorpe & Morton, 1871; aluminium and sulphuric acid, Gad-Andresen, 1923) which have been used for this purpose are satisfactory if the ammonia is to be separated by diffusion, owing to the evolution of hydrogen which causes pressure to develop in the Cavett flask. Attempts to carry out the reduction using ferrous hydroxide and titanous salts gave very poor recoveries of ammonia.

Raney nickel (semi-colloidal nickel prepared by the action of strong alkali on nickel-aluminium alloy) has been used by Van Dalen (1951) as a catalyst in the hydrogenation of macro amounts of nitrate to ammonia. Since the catalytic nickel contains up to 120 ml. H/g (Mozingo, Wolf, Harris & Folkes, 1943) it was thought that microgram quantities of nitrate could be reduced to

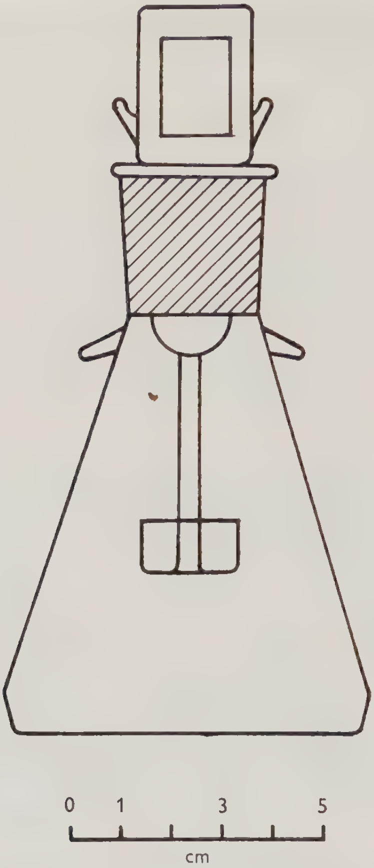

Fig. 1. A Cavett flask as used for the microdiffusion of ammonia.

TABLE 1. RECOVERY OF AMMONIA FROM 50 ML. OF SEA WATER CONTAINING ADDED AMMONIUM SULPHATE AND 10 ML. OF METABORATE BUFFER DIFFUSED AT 70° C FOR 20 H.

μg NH_4^+-N		Mean recovery (%)
Added	Found	
0·0	0·0	—
2·5	1·7 1·7 1·8	69
5·0	3·5 3·6 3·6 3·7	72
10·0	7·2 7·5	73

ammonia by the action of its adsorbed hydrogen. This proved to be the case. It was found that the reduction proceeded much more rapidly in the presence of ethylenediamine-tetraacetic acid (EDTA) which also served to reduce the quantity of magnesium and calcium salts precipitated from the sea water at the pH of 10·5 used in the reduction and diffusion.

COLORIMETRIC DETERMINATION OF AMMONIA

The advantages of the phenate-hypochlorite method over the Nessler method for the colorimetric determination of ammonia have been enumerated by Riley (1953). Crowther & Large (1956) have found that the indophenol-blue colour develops rapidly at room temperature if the sodium phenate reagent is made up in an aqueous mixture of acetone and methyl alcohol instead of water.

TABLE 2. EFFECT OF VARIATION OF VOLUMES OF SODIUM PHENATE AND SODIUM HYPOCHLORITE REAGENTS ON OPTICAL DENSITIES OF SOLUTIONS MEASURED AT 625 Mμ IN A 1 CM CELL (10 μG NH_4^+-N IN FINAL VOLUME OF 10 ML.)

ml. of hypochlorite*	0·5	1·0	1·5	2·0	2·5	3·0
0·5 ml. of phenate†	0·387	0·340	0·280	0·234	0·185	0·165
1·0 ml. of phenate†	0·372	0·380	0·373	0·370	0·350	0·328
1·5 ml. of phenate†	0·273	0·320	0·337	0·370	0·365	0·352
2·0 ml. of phenate†	0·163	0·280	0·310	0·340	0·355	—

* 0·9 % available chlorine.

† In methanol containing acetone.

In order to find the optimum conditions for the determination of ammonia using Crowther's reagent, determinations were carried out using 10 μg NH_4^+-N and varying the amounts of both sodium phenate (prepared according to Crowther & Large, 1956) and sodium hypochlorite added. The optical densities of the solutions were measured at 625 mμ after 1 h. The results, which are given in Table 2, indicated that 1 ml. of sodium phenate and 1·5 ml. of sodium hypochlorite were the most suitable volumes. Under these conditions, which were adopted for all subsequent work, the maximum optical density was attained after 45 min at 20° C, and remained constant for a further 60 min, after which time slow fading occurred.

A calibration curve was prepared using known amounts of ammonium salt (Fig. 2); it indicated that Beer's law is obeyed up to a concentration of 1·5 μg NH_4^+–N/ml. (in a final volume of 10 ml.). Above this concentration the change in optical density per unit weight of NH_4^+–N is considerably reduced. Replicate determinations (6) carried out with 5 and 10 μg NH_4^+–N gave mean optical densities (less blank) of 0·190 ± 0·001 and 0·377 ± 0·002.

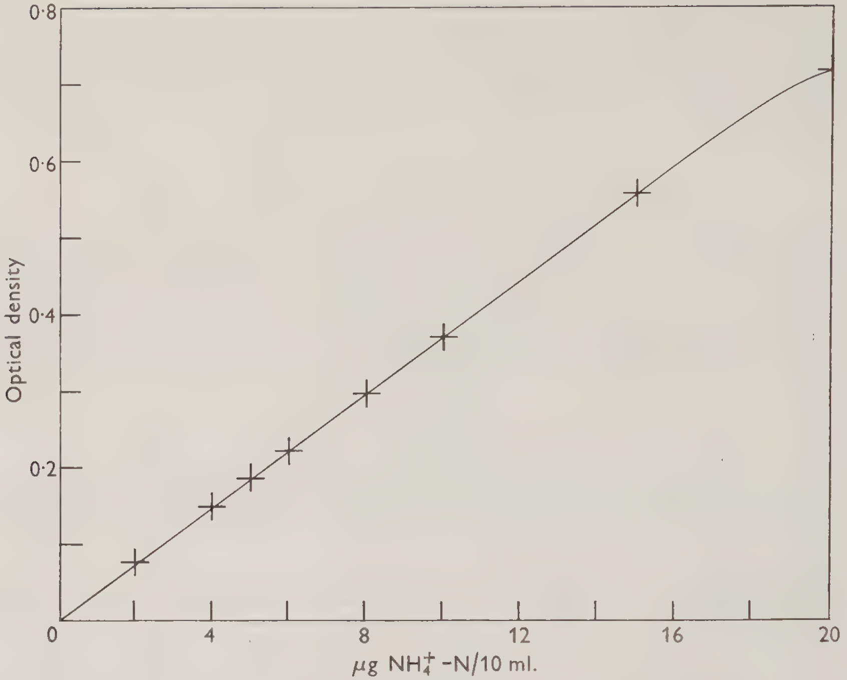

Fig. 2. Calibration chart of the method for the determination of ammonia in sea water, prepared by using known amounts of ammonia. Optical density at 625 mμ using a 1 cm cell.

METHOD

All measurements of optical density were made with a Unicam S.P. 500 spectrophotometer using 1 cm glass cells, against distilled water in the compensating cell.

REAGENTS

All reagents and solutions must be made up with water which has been freshly distilled from dilute sulphuric acid in an all-glass still.

1. *Metaborate buffer*

Dissolve 2·55 g of sodium metaborate and 2·0 g of sodium hydroxide in distilled water, dilute to 100 ml.

2. *Raney nickel*

Weigh out *c.* 5 g of powdered nickel-aluminium alloy, (50/50) and add it gradually to a 250 ml. beaker containing a solution of 10 g of sodium hydroxide in 70 ml. of distilled water. After the addition has been completed, heat the covered beaker on a hot plate for 30 min, when all effervescence should have ceased. Wash the finely divided nickel with hot water by decantation, until the washings are free from alkali. Preserve it under water and do not allow it to become dry. Fresh Raney nickel should be prepared at least weekly.

Caution: care should be taken when disposing of waste Raney nickel as it is pyrophoric when dry.

3. *EDTA reagent*

Dissolve 4 g of ethylenediamine-tetraacetic acid (disodium salt) in 100 ml. of distilled water.

4. *Sodium hydroxide* (2 N)

Gently boil a solution of 8 g of sodium hydroxide in 60 ml. of water for a few minutes. When cool, dilute to 100 ml.

5. *Hydrochloric acid* (0·02 N)

Dilute 2 ml. of concentrated hydrochloric acid to 1000 ml.

6. *Sodium phenate reagent*

(*a*) Phenol solution (solution A). Dissolve 62·5 g of phenol B.P. in methyl alcohol, add 20 ml. of acetone and dilute to 100 ml. with methyl alcohol. The reagent, which should be prepared each week, must be kept in a refrigerator.

(*b*) Sodium hydroxide (solution B). Dissolve 27 g of sodium hydroxide A.R. in *c.* 60 ml. of distilled water, boil gently for a few minutes to remove ammonia, cool and dilute to 100 ml.

For use mix 20 ml. portions of solutions A and B and dilute to 100 ml. The mixed reagent should be prepared freshly as required.

7. *Sodium hypochlorite reagent*

The reagent solution is prepared by dilution of commercial sodium hypochlorite solution and contains 0·9 g of available chlorine per 100 ml. The strength of the stock solution should be checked periodically.

STANDARD SOLUTIONS

1. *Ammonium sulphate*

Weigh out 0·4716 g of ammonium sulphate A.R., dissolve in water and dilute to 1000 ml. This solution, which contains 100 μg NH_4^+-N/ml. is used for the preparation of the working solution containing 2·5 μg. NH_4^+-N/ml.

2. *Potassium nitrate*

Prepare a solution of potassium nitrate containing 10 μg NO_3^--N/ml. by dissolving 0·0722 g of the A.R. grade salt in water and diluting to 1000 ml. Prepare from this a working solution containing 2·5 μg NO_3^--N/ml.

3. *Sodium nitrite*

Dissolve 0·1099 g of recrystallized silver nitrite in about 10 ml. of boiling water, and add while still hot *c*. 0·10 g of sodium chloride. Shake until the silver chloride flocculates, set aside in the dark until the solution has cleared. Dilute to 1000 ml. From this solution, which contains 10 μg NO_2^--N/ml., prepare a working solution containing 2·5 μg. NO_2^--N/ml.

TREATMENT OF FLASKS

Wash all Cavett flasks and graduated flasks with concentrated hydrochloric acid. Rinse with distilled water, then with 0·1 N sodium hydroxide and again with water, allow them to drain. Lubricate the upper part of the ground surface of the Cavett flask stoppers with a mixture of equal weights of paraffin wax (congealing point 49° C) and medicinal paraffin.

DETERMINATION OF AMMONIA IN SEA WATER

Pipette 50 ml. of filtered sea water into a 150 ml. conical flask fitted with a B24 socket, add 2 ml. of 4% EDTA reagent and raise to boiling on the hot plate. Remove from the heater and add *c*. 2 ml. of sodium metaborate buffer from a fast running pipette. Immediately close the flask with a lubricated Cavett stopper (Quickfit and Quartz Catalogue No. BC3CH), the cup of which contains 1 ml. of 0·02 N hydrochloric acid. Gently shake the stoppered flask and then heat in an oven at 70° C. After 24 h remove the flask from the oven and allow to cool for a few minutes. Transfer the hydrochloric acid in the cup to a 10 ml. graduated flask by means of a drawn-out glass tube fitted with a rubber teat. Wash the cup well with distilled water and transfer the washings to the graduated flask. To the combined solutions add 1 ml. of sodium phenate reagent and 1·5 ml. of sodium hypochlorite solution, dilute to volume and mix thoroughly. After 45–60 min measure the optical density at 625 mμ. Run a reagent blank on 10 ml. of distilled water in the same manner. Calibrate the method by carrying out a determination on 50 ml. of the same sea water to which 5 μg NH_4^+-N has been added.

DETERMINATION OF TOTAL IONIC INORGANIC NITROGEN IN SEA WATER

Pipette 50 ml. of sea water into a 150 ml. conical flask fitted with a B24 socket, add from a small scoop *c*. 0·2 g of Raney nickel and 1 ml. of 4% EDTA reagent. Heat the solution on a hot plate and allow to boil gently for 15 min. Remove from the heater and add rapidly 2 ml. of 2 N sodium hydroxide. Immediately close the flask with a well-lubricated Cavett stopper, the cup of which contains 1 ml. of 0·02 N hydrochloric acid. Place in an oven at 70° C to allow the ammonia to diffuse. After 24 h determine the ammonia as described above.

Carry out a blank determination on 10 ml. of distilled water and a calibration run on the same sea water, to which has been added 5 μg NO_3^- or NO_2^--N.

Great care must be taken to avoid contamination by extraneous nitrogen compounds during the determination; in particular, the apparatus should not be handled more than is absolutely necessary.

It is important also to take approximately the same amount of Raney nickel in each determination and in the blank, since it always yields small amounts of ammonia during the diffusion.

RESULTS

In order to test the recovery of nitrogen from sea water, samples of sea water very low in inorganic nitrogen were enriched by the addition of known amounts of nitrate, nitrite, or ammonium-nitrogen. These samples were then analyzed for total nitrogen as described on p. 166. The results are shown in Table 3. They show that the reduction is complete since a recovery of 73 ± 4 % of nitrate, nitrite and ammonium-nitrogen can be obtained using a diffusion time of 24 h. Higher recoveries can be obtained with longer diffusion periods, but the results are less reproducible. Using the recommended conditions, replicate determinations (7) were carried out on a sample of Irish Sea water; they showed that the water contained 240 μg N/l. with a coefficient of variation of 2·5 %.

TABLE 3. RECOVERY OF INORGANIC NITROGEN FROM SEA WATER

Nitrogen added	Nitrogen recovered (μg N)								Average recovery	Percentage recovery
Sea water only	0								0	—
2·5 μg NH_4^+-N	1·9	1·9	1·7	1·8	1·7	2·0	2·0		1·86	74
5·0 μg NH_4^+-N	3·5	3·4	3·3	3·6	3·5	3·6			3·48	70
10·0 μg NH_4^+-N	7·6	7·6	7·5	7·5	7·3	7·7			7·53	75
2·5 μg NO_3^--N	2·0	1·7	1·8	2·1	2·1	1·9			1·92	77
5·0 μg NO_3^--N	3·5	3·7	3·7	3·6	3·7				3·64	73
10·0 μg NO_3^--N	7·1	7·3	7·5	7·7	7·6	7·7	7·2	7·3	7·42	74
2·5 μg NO_2^--N	1·5	1·7	1·8						1·69	68
5·0 μg NO_2^--N	3·8	3·9	3·3	3·7	3·4	3·4	3·9		3·62	72
10·0 μg NO_2^--N	7·5	7·3	7·6	7·9					7·58	76
									Mean	73 %

In order to investigate the possible interference which might be caused by the breakdown of organic nitrogen compounds during the diffusion, the determination was carried out in the presence of a number of typical organic nitrogen compounds. No interference was experienced using 20 μg of DL-α-alanine, L-arginine hydrochloride, cystine, glutamic acid, urea, or choline chloride.

SUMMARY

Microdiffusion using a Cavett flask has been employed instead of vacuum distillation for the separation of ammonia from sea water; diffusion is approximately 75% complete after 24 h at 70° C. The recovered ammonia is determined by a modification of the phenate-hypochlorite method.

Raney nickel in the presence of ethylenediamine-tetraacetic acid has been used for the reduction of nitrate and nitrite to ammonia. The latter is separated by microdiffusion and determined colorimetrically. The method showed a coefficient of variation of 2·5% on a sample of sea water containing

$$240\,\mu g\ \mathrm{NO_2^- + NO_3^- + NH_4^+ - N/l.}$$

No interference was encountered from a variety of organic nitrogen compounds.

REFERENCES

Brandt, K., 1927. Stickstoffverbindungen im Meere I. *Wiss. Meeresunters.* (Abt. Kiel), Bd. 20, p. 212.

Cavett, J. W., 1937. The determination of alcohol in blood and other body fluids. *J. Lab. clin. Med.*, Vol. 23, pp. 543–6.

Conway, E. J., 1950. *Microdiffusion Analysis and Volumetric Error*, 3rd ed. 391 pp. London: Crosby Lockwood.

Crowther, A. B. & Large, R. S., 1956. Improved conditions for the sodium-phenoxide-sodium hypochlorite method for the determination of ammonia. *Analyst*, Vol. 81, p. 64.

Gad-Andresen, K. L., 1923. A method for quantitative determination of ammonia, nitrate and nitrite, together with other nitrogenous compounds in sea-water. *Publ. Circ. Cons. Explor. Mer*, No. 82, pp. 14–17.

Krogh, A., 1934. A method for the determination of ammonia in water and air. *Biol. Bull., Woods Hole*, Vol. 57, pp. 126–31.

Mozingo, R., Wolf, D. E., Harris, S. A. & Folkes, K., 1943. Hydrogenolysis of sulphur compounds by Raney nickel catalyst. *J. Amer. chem. Soc.*, Vol. 65, p. 1013.

Raben, E., 1905*a*. Weitere Mitteilungen über quantitative Bestimmungen von Stickstoffverbindungen und gelöster Kieselsäure im Meerwasser. *Wiss. Meeresunters.* (Abt. Kiel), Bd. 8, pp. 279–80.

Raben, E., 1905*b*. Über quantitative Bestimmung von Stickstoffverbindungen im Meerwasser. *Wiss. Meeresunters.* (Abt. Kiel), Bd. 8, pp. 81–98.

Raben, E., 1910. Dritte Mitteilung über quantitative Bestimmungen von Stickstoffverbindungen und von gelöster Kieselsäure im Meerwasser. *Wiss. Meeresunters.* (Abt. Kiel), Bd. 11, pp. 305–19.

Raben, E., 1914. Vierte Mitteilung über quantitative Bestimmungen von Stickstoffverbindungen im Meerwasser und Boden sowie von gelöster Kieselsäure im Meerwasser. *Wiss. Meeresunters.* (Abt. Kiel), Bd. 16, pp. 207–29.

Riley, J. P., 1953. The spectrophotometric determination of ammonia in natural waters, with particular reference to sea-water. *Anal. chim. acta*, Vol. 9, pp. 575–89.

Thorpe, T. E. & Morton, E. H., 1871. Über das Wasser des Irländischen Meeres. *Liebig's Ann. Chem.*, Bd. 158, dp. 122–31.

Van Dalen, E., 1951. Schnelle Bestimmung von Nitrat in lösung mittels katalytischer Reduktion mitt Wasserstoff. *Anal. chim. acta*, Vol. 5, pp. 463–71.

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THE TOXIN FROM *GYMNODINIUM VENEFICUM* BALLANTINE

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(Text-figs. 1–8)

One of the authors (Ballantine, 1956) has recently described a new species of toxic dinoflagellate (*Gymnodinium veneficum*). Interest in the toxicity was stimulated by Bainbridge's observations (1953) on this species and by the well-known problem of mass mortality associated with 'red tide' outbreaks of poisonous flagellates. Much has been published on this latter subject, for which extensive bibliographies are available in the papers of Brongersma-Sanders (1948) and Hayes & Austen (1951).

Dinoflagellates in particular have long been associated with marine fish mortalities (see Davis, 1948, on *Gymnodinium brevis*, and the review by Nightingale, 1936) and with the problem of paralytic shellfish poisoning (Sommer, Whedon, Kofoid & Stohler, 1937, *Goniaulax catenella*; Koch, 1939, *Pyrodinium phoneus*; and Needler, 1949, *Goniaulax tamarensis*).

The published work on the mode of action of dinoflagellate toxins has been mainly concerned with the practical aspects linked with the cause, effects and prevention of paralytic shellfish poison, and has been directed towards mammalian and amphibian tissues (e.g. Kellaway, 1935; Fingerman, Forester & Stover, 1953), with attempts to ascertain the chemical nature of the poisons and with bio-assay. As there is no 'red tide' problem here, we are not concerned with the essential practical preventative approach and have attempted, over a wide field, to determine the actual site and mode of action of the toxin in marine animals, in addition to trying to extract it and find the effect on whole animals.

All our experiments have made use of a uni-algal culture of *Gymnodinium veneficum* grown in Erdschreiber culture solution (Plymouth formula).[1] These cultures are not bacteria-free, but repeated attempts to grow the bacteria from these cultures in a variety of marine media have always resulted in non-toxic cultures. In view of this we feel confident that the toxin is either a product of the *Gymnodinium* itself or possibly a product of the action of bacteria on a waste substance produced by the *Gymnodinium*. This latter possibility does not seem probable, as the substance is an exotoxin secreted into the water, and whereas about 90% of the total toxin in any culture is present in the supernatant fluid, on a volume to volume basis the cells contain about 200–500 times as much toxin as the water. In addition, when the bacterial population of a culture becomes high the toxicity decreases.

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[1] Erdschreiber. Filtered sea water 1 l., pasteurized. Soil extract (*w*/*v*) 50 ml., $NaNO_3$ 0·3 g, $Na_2HPO_4 . 12H_2O$ 0·02 g.

In this research we had assistance and advice from many people. We cannot express our thanks to all personally, but in particular we mention the folowing: Dr Mary Parke for providing large volumes of *Gymnodinium* culture; Dr G. Y. Kennedy and his assistants of Sheffield University who have carried out all experiments using mice; Dr N. R. Stephenson of the Department of National Health and Welfare of Canada for a supply of paralytic shellfish poison; Dr J. P. Quilliam of St Bartholomew's Hospital, London, for carrying out experiments with isolated ganglia; Dr D. B. Carlisle of this laboratory for assistance with experiments on isolated hearts and much helpful discussion; Dr E. D. S. Corner, International Paints Research Fellow at Plymouth, for experiments on respiration rates, for a great deal of help and advice on extracttion techniques and for continued encouragement; and to the Director and staff of the Plymouth Laboratory, especially workshop staffs, for their unfailing interest in this work, and the ready production of apparatus when required.

EXTRACTION

In studies on the effect of the *Gymnodinium* toxin on whole marine animals, whole cultures of the flagellate or supernatant water after the removal of the cells were used. The specific action of the toxin, however, could be investigated only on isolated tissues from animals and the use of sea water as a bathing medium severely limits the range of material. Extraction of the toxin in a more concentrated form free from salt therefore became necessary.

Most of the work on paralytic shellfish poison has been based on extraction from the digestive glands of poisonous but living shellfish. The procedure generally adopted in this extraction has been to mince the digestive glands and extract the toxin with acidified alcohol (4 ml. conc. HCl/l. alcohol), centrifuge, evaporate to dryness and wash with chloroform (Müller, 1935). The method has since been extended by other workers and purer extracts have been produced (Sommer & Meyer, 1937; Sommer, Monnier, *et al.* 1948; Sommer, Riegel, *et al.* 1948; and Riegel, *et al.* 1949*a*).

In view of the concentration of toxins from *Goniaulax* spp. by shellfish we fed *Mytilus edulis* with cultures of *Gymnodinium veneficum* in an attempt to obtain a similar effect. Strong cultures were lethal within a day and more dilute cultures eventually killed the *Mytilus* without any significant concentration of the toxin by the animal.

It was therefore necessary for us to extract the toxin from cultures. The methods of Sommer *et al.* referred to above have been extended to extraction from the cells of *Goniaulax catenella* (Sommer, Whedon, *et al.* 1937; Riegel *et al.* 1949*b*). We followed this method using centrifuged cells of *Gymnodinium veneficum* and dissolved the solid residue in the appropriate bathing media for experiments on isolated tissues. Extracts prepared by this means were also dissolved in sea water, and it was found that immersion of fish in this solution did not give the same symptoms as the original culture (see p. 176). The cells were therefore extracted with neutral alcohol and under these conditions the characteristics of the original culture were completely retained. In all our work we find that 'acid extraction' alters the toxin and we have employed 'neutral extraction' in all cases except when stated. Hashimoto & Migita (1952) have noted a similar effect.

Extraction from cells only is very wasteful as most of the poison remains in the supernatant fluid, and large volumes of culture were needed in order to obtain significant quantities of cells. The supernatant fluid contains about 90% of the toxin in most cultures, but the problem is to separate it from the salt. Adsorption on carbon columns was attempted. Owing, however, to the large amount of salt present it was necessary to use a very active charcoal to adsorb the toxin in reasonable amounts. Elution from such carbon was very difficult; hot ethyl alcohol containing 5% pyridine or hot dioxan were the only successful eluants. These were removed by evaporation under reduced pressure. Owing to the variability between batches of carbon and to the difficulty of removing final traces of solvents dialysis was tried. Cell-free culture fluid was dialysed in tubes and the salt had gone in 6 h, leaving behind the toxin which was then evaporated to dryness under reduced pressure. Very little toxin is lost through the membrane indicating that the toxin molecule is large, probably of mol.wt. > 1000. By this process an active extract was obtained, although the product was contaminated by impurities from the soil extract used in growing the cultures. Work on the purification of these extracts will be attempted, but it is obvious even at present that this is a very potent poison.

EXPERIMENTS WITH WHOLE ANIMALS

Throughout this series of experiments marine animals were taken from the aquarium sea water and placed either in whole cultures of *Gymnodinium veneficum* or in the supernatant culture fluid from which the cells had been removed. Experiments with other non-toxic flagellates have shown that this treatment produces no ill-effects.

Various animals have been used (see Table 1) and they appear to fall into three main groups, as follows:

(A) *Not affected.* Animals of only one group, the Polychaeta, have been found to be completely unaffected by the toxin. Specimens of *Nereis diversicolor* and *Arenicola marina* survive in cultures apparently indefinitely. With

TABLE 1

Animal	Mode of treatment	Reaction group (see p. 171)	Time to death
Coelenterata			
Calliactis parasitica	Imm., Imm. + inj.	B	2–3 days
Anemonia sulcata	Imm., Imm. + inj.	B	3–4 days
Ephyrae of *Aurelia aurita*	Imm.	C	< 1 min
Annelida: Polychaeta			
Nereis diversicolor	Imm.	A	—
	Imm. + inj.	A	—
Nereis diversicolor	Imm. + inc.	A	—
Arenicola marina	Imm.	A	—
Arthropoda: Crustacea			
Calanus finmarchicus (see Marshall & Orr, 1955)	Imm.	B	1–2 days
Tigriopus fulvus	Imm.	B	1–3 days
Hemimysis lamornae (see Bainbridge, 1953)	Imm.	B	2–3 days
Macromysis flexuosus	Imm.	B	3–4 days
Palaemon serratus	Imm.	B	> 4 days
Eupagurus bernhardus	Imm.	B	2–3 days
Carcinus maenas	Imm.	B	> 4 days
Cancer pagurus	Imm.	B	3 days
Mollusca			
Mytilus edulis	Imm.	B	< 3 days
M. galloprovincialis	Imm.	B	< 3 days
Pecten maximus	Imm.	C	1 h
Lasaea rubra	Imm.	B	> 1 day
Buccinum undatum	Imm.	C	1 h
Aplysia punctata	Imm.	C	1 h
Eusepia officinalis (cuttle fish)	Imm.	C	3 h
Echinodermata			
Asterias rubens	Imm.	B	3–5 days
Ophiothrix fragilis	Imm.	B	< 3 days
Ophiocomina nigra	Imm.	B	< 3 days
Tunicata			
Ciona intestinalis	Imm.	B	3 days
Cephalochordata			
Amphioxus lanceolatus	Imm.	C	45 min
Pisces			
Scyllium canicula (2 ft.) (dogfish)	Imm.	C	3 h
Gadus pollachius (3 in.) (pollack)	Imm.	C	18 min
Blennius gattorugine (blenny)	Imm.	C	< 45 min
Trachinus vipera (lesser weaver)	Imm.	C	30 min
Gobius niger (goby)	Imm.	C	5–15 min
G. virescens (goby)	Imm.	C	5–10 min
Pleuronectes platessa (5 in.) (plaice)	Imm.	C	30 min
Ctenolabrus rupestris (wrasse)	Imm.	C	15–20 min
Amphibia			
Rana temporaria (frog)	Inj.	C	Strong rapid paralysis kills in *c.* 30 h
Mammalia			
Mus musculus (mouse)	Inj.	C	2–4 min

Controls using sea water and non-toxic cultures were made in all experiments, and the control animals survived much longer and were still healthy when the test animals had all died.

Imm., immersion in culture; inj., injection of extract; inc., incision.

Nereis some animals were injected and others cut down the ventral surface. They were then placed in cultures but showed no ill effects.

(B) *Slowly affected.* In this category are various animals including members of the Coelenterata, Crustacea, Mollusca, Echinodermata and Tunicata. It is felt that with all these, except the Mollusca, which can close their shells protectively, the reason for the slow effect of the toxin is due to the fact that the large toxin molecule cannot penetrate the animal; for Dr Sheina M. Marshall (personal communication) reports that *Calanus* which ate a large number of *Gymnodinium* from strong actively growing cultures died within 18 h, whereas poor feeders survived some days. We have found that this applies also to the other crustaceans, except *Eupagurus* where the increased sensitivity may be associated with the soft body.

Ophiothrix and *Ophiocomina* both fragment when immersed in *Gymnodinium* cultures, and the arms are finally broken up into short segments leaving the body which then dies.

In the molluscs of group B, i.e. *Lasaea* (see Ballantine & Morton, 1956) and *Mytilus*, the mechanism for prevention of entry of the toxin is simply shell closure. Both are intertidal bivalves capable of withstanding long exposure to unfavourable conditions and they close up when immersed in this culture. Eventually, however, sufficient poison enters to kill them. In all animals of this group, isolated excitable tissues are quickly blocked by the toxin (see p. 179).

(C) *Rapidly killed.* This last group is by far the most interesting and with it most of the work on whole animals has been carried out. Into this section fall molluscs without an adequate shell closure mechanism, *Amphioxus*, all the fish, frogs and mice—the last two after injection. In the marine animals the primary site of entry of the toxin is probably through the gills and not orally, as the supernatant culture fluid from which all cells have been removed is as quickly lethal as the whole culture, even with continuous aeration, and evidence from feeding experiments with mice suggests that such rapid and lethal penetration of the toxin cannot take place through the gut.

Gobius virescens was used as the test animal for comparison of the toxicity of cultures and extracts because of its availability and sensitivity to the toxin. A strong culture kills the fish in 5–6 min, the death time being prolonged as the toxin strength is reduced, and a bio-assay method has been worked out on this basis (Fig. 1). The symptoms shown by all fish when immersed in cultures, supernatant fluid from cultures, cells resuspended in sea water, and all toxic extracts produced at pH 7–8·5, were identical. The immediate reaction of the fish was a violent attempt to swim away from the unpleasant medium. In the goby this violent swimming, either forwards or backwards, continued for about 2 min and then subsided. Intense vasodilation and a strong expansion of the chromatophores to produce a marked colour pattern occur at this stage. At the same time the balance control mechanism is upset, and the fish floats on its side or even upside down. Breathing slows considerably from a normal rate of 75–90/min to about 45/min after 2 min. Soon after immersion breathing is interrupted by a spasmodic violent 'vomiting reaction', which continues until death. Breathing continues to slow down, turning to irregular gasping at long intervals (up to 20 sec) and there is a lack of sensory response.

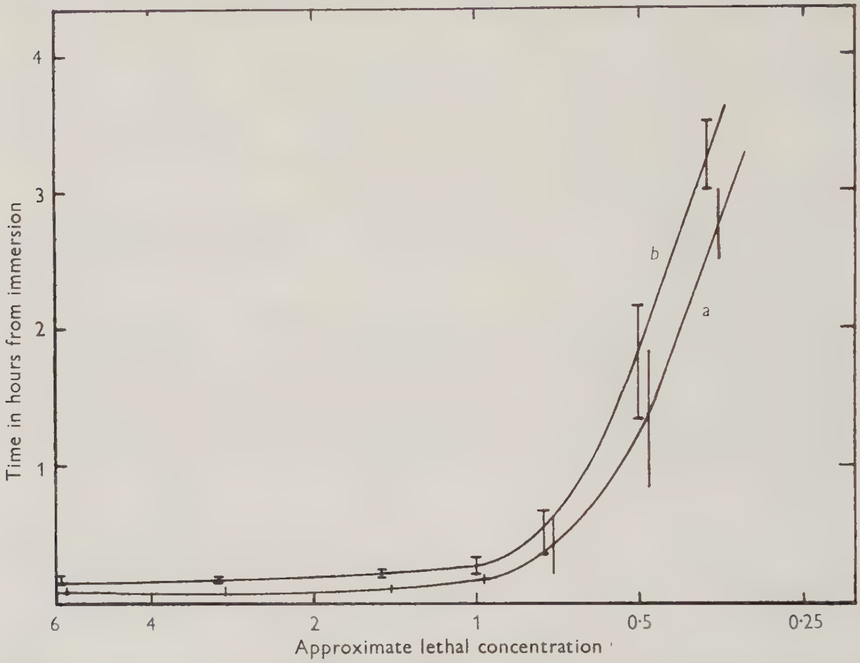

Fig. 1. Assay curve for toxin strength using gobies. Curve *a*, time to complete loss of balance in fish. Curve *b*, time to death. Extremes shown by vertical lines. Concentration in arbitrary units defined in text.

Reaction to touch or prodding disappears progressively from the head towards the tail, the base of the tail retaining sensitivity longest. Death occurs in quiescent paralysis, with the gills extended, apparently due to some sort of respiratory failure, but is preceded by a few irregular bursts of violent activity. If removed to clean sea water before the balance is upset the fish will recover; but there is no survival once the balance has gone.

The bio-assay technique we have developed using gobies is simple but only approximate. Fig. 1 shows the graphs obtained by plotting time to complete loss of balance (*a*) and time to death (*b*) against dose in arbitrary units. By using these two criteria it is possible to find an approximate lethal concentration per ml. of the sea water in which fish are immersed by using two or three different dilutions. This 'approximate lethal concentration' may be defined as the amount of toxin per ml. which causes permanent loss of balance in 8–15 min and death in 10–20 min. A point which must be made about this assay is the fact that these times are constant with the gobies used (*G. virescens* and *G. niger*, where the loss of balance is interpreted as that stage when the fish can no longer right itself when inverted) regardless of the size of the goby. Fish from 17 mg to 7 g have been tested and body size has no effect on death time when a given dose of toxin is used. This is probably due to the fact that the toxin penetrates through the gills and the active gill surface area is roughly proportional to the size of the fish within a species. The average lethal concentration per ml. of a strongly toxic culture is about 6 units.

Because of its large size further experiments were carried out on the dogfish, and general symptoms were the same. Just before death (at the completely quiescent stage) the animal was removed and cut open. The heart was beating strongly, and there was no sign of haemolysis in blood samples taken. Electrical stimulation of the body muscles showed that these still responded to a direct stimulus. Although the muscles can themselves be poisoned by the toxin (p. 182), death is not due to failure of the muscles, and must result from a blockage in the nervous system.

Invertebrates of group C die with very few symptoms. Ephyrae of *Aurelia* pulsated about 5 times and then sank to the bottom fully contracted. Quick removal into clean sea water gave an eventual recovery in most cases. Molluscs died with their muscles relaxed, the bivalves with shells gaping.

During these experiments we have noted that, when agitated, the cultures produce an irritant vapour or mist (probably a suspension of droplets in air) which gives discomfort to throat and nose, of the type associated with an incipient cold. This phenomenon has also been observed as being associated with red tides in America (Woodcock, 1948; Ingle, 1954). One member of the staff of this laboratory, who is extremely susceptible to hay fever and allergic to many types of dust, is immediately and strongly affected by this mist. Since this type of reaction is associated with histamine release, several experiments were carried out on fish exposed to a variety of drugs. The histamine releaser '48–80' in sea water at strengths up to 100 μg/ml. has no effect at all on gobies. Histamine itself at 2·5 mg/ml. is not lethal but disturbs the animal and causes complete contraction of the chromatophores (greyish white) in contrast to the expansion in *Gymnodinium*. The addition of the antihistamine drug Phenergan at 2·5 μg/ml. to histamine-treated animals restored them to normal in a few minutes. On the other hand, Phenergan alone at 5 μg/ml. is lethal to gobies in 5 h. Phenergan gave no protection at all to gobies in *Gymnodinium* culture, and in fact hastened death. It therefore seems that there is no direct relation between the action of histamine and the toxin.

Evidence of protection against the toxin comes from two types of experiment: previous treatment in non-toxic flagellates, and addition of cholesterol. When gobies have been kept in cultures of non-toxic flagellates (e.g. *Isochrysis galbana* Parke) for 2 weeks, removed into clean sea water to clear the gills of any possible clogging and then placed in *Gymnodinium* culture, time to death was prolonged from 15 min (control) to 2 hr. This protection was only slight. The influence of cholesterol was more dramatic, however. Cholesterol ground to a fine suspension in alcohol was added to sea water containing *Gymnodinium* culture. It was found that with a concentration of toxin of about 4 lethal units/ml (see Fig. 1), complete protection was provided if 20 mg. cholesterol was added per 100 ml sea water. Smaller doses prolonged the time to death. The actual amount of cholesterol which went into solution was uncertain.

Whereas extracts made at or near neutrality gave the same symptoms as the original culture, those made at pH 4, extracting from the centrifuged cells or from carbon columns with acidulated methyl alcohol (as described by Medcof *et al.*, 1947; and Müller, 1935), proved non-lethal to fish. The acid extract has a pronounced excitatory effect, does not slow breathing, and shows some resemblances to the action of acetylcholine. This difference in action between neutral and acid extracts is very marked. A similar observation has been made by Hashimoto & Migita (1952) on extracts from mollusc livers. Similar symptoms to these are shown by fish immersed in large doses of scallop liver extract (20 mg/100 ml.) which, if lethal, kills only after prolonged exposure (>1 day).

All the experiments on mice reported below were done at the Cancer Research Department of Sheffield University by Dr G. Y. Kennedy and his assistants. Extracts were given to mice either orally or by intraperitoneal injection. Injection of neutral extracts in lethal doses caused death within 2 min, with immediate prostration and no other symptoms. There were no post-mortem aberrations. With sublethal doses the mice looked 'thoughtful' and slightly depressed, becoming moribund as the dose was increased. Eventual recovery was complete. Injection of the acid extract produced a slight excitation but was not lethal in the doses given.

We have been fortunate in obtaining a sample of lyophilized scallop liver extract containing the acid extract of paralytic shellfish poison from Dr N. R. Stephenson of the Department of National Health and Welfare of Canada (see also Stephenson *et al.*, 1955). This was also administered to mice during the series of tests. Injection of lethal doses produced prostration, and sublethal doses gave excitation. Oral administration also produces excitation of the animals, and is less toxic than injection. The *Gymnodinium* extracts were not toxic when administered orally, but again caused excitation. Thus it would appear that the neutral extract undergoes a change during digestion, probably due to the acid nature of the gastric juices, giving symptoms similar to those produced by acid extracts of *Gymnodinium* and by scallop liver extracts.

The evidence from fish experiments suggesting that death is due to respiratory failure is supported by injection of frogs. A quantity of neutral extract sufficient to kill a mouse twice the weight was injected into the dorsal lymph sac of frogs. This caused immediate and complete paralysis, and the animals remained moribund for about 2 h. Some recovery then appeared to occur, but the animals died in 24–48 h. There was no sign of breathing over this period, but the oxygen supply through the skin must have been adequate to sustain life for this length of time. Prinzmetal, Sommer & Leake (1932) have also noted this greater resistance of frogs to paralytic shellfish poison.

Although death in vertebrates seems to be due to failure of the respiratory system to provide an adequate oxygen supply there is no doubt that the poison acts by blocking the nervous system.

EXPERIMENTS ON ISOLATED TISSUES

These were carried out to show the actual site of action of the toxin, and form a progressive series as information was accumulated. Unless it is otherwise stated, the tissues were treated with either the supernatant fluid from cultures if sea water was a suitable bathing medium, or neutral extracts of the toxin dissolved in an appropriate bathing fluid. 'Acid extracts' were used only in a few experiments, and a distinct difference in action was found in all of them, after which 'acid extraction' was abandoned in this project. The experiments will be grouped together under a series of general headings and for brevity only the salient points emerging will be discussed.

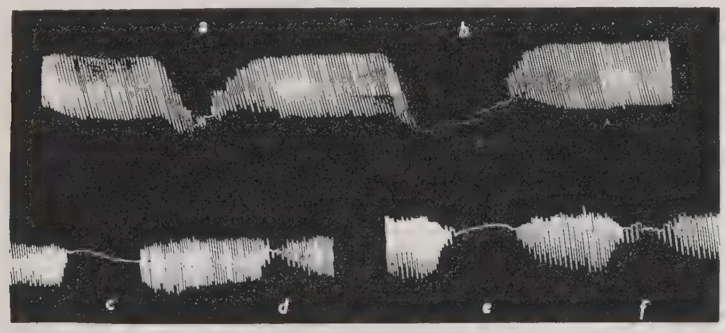

Fig. 2. Action of *Gymnodinium* toxin on perfused *Buccinum* heart. Contraction of heart (systole) downwards; perfusion fluid sea water; temp. 15° C. (*a*) Original culture diluted twofold; (*b*) neutral extract; (*c*) 'acid extract'; (*d*) 'acid extract' at half previous strength; (*e*) and (*f*) acetylcholine at concentrations of 5×10^{-7} and 2×10^{-7} respectively.

Isolated hearts.

Hearts from various animals were isolated and perfused. Recording was on a kymograph with a normal heart lever. Frog, *Loligo* and *Buccinum* hearts were all arrested in systole by the toxin, in contrast to the actions of acetylcholine and the 'acid extract', both of which cause arrest in diastole (Fig. 2).

Buccinum heart appears to be quite sensitive to the toxin and may be suitable for a comparative assay technique. The disadvantage of *Buccinum* heart when isolated and suspended is that the auricle is extremely friable, and the ligatures necessary for cannulation frequently sever it. A more reliable preparation results from cannulation of the heart *in situ*, via the efferent branchial vein. If a cannula with a long tip is used there is no need to ligate this vein. A small amount of citrate added to the perfusion fluid (sea water) prevents coagulation of the residual blood in the heart.

With *Maia* heart the 'acid extract' increases the heart rate whereas the neutral extract slows it.

Frog rectus abdominus muscle

Because of the similarity in action of the 'acid extract' and acetylcholine on isolated hearts we investigated the effect of our extracts on frog rectus abdominus muscle. The muscle was mounted in a chamber and connected to a simple lever which recorded on a kymograph. Bathing solutions were run into the chamber as necessary. The response to acetylcholine was first measured. Application of the 'acid extract' of the toxin caused a slow, steadily increasing contracture, but on the addition of acetylcholine the muscle contracted to the same final level as with acetylcholine alone. Relaxation of the contractured muscle is very slow. The neutral extract induces no contracture, but reduces the response of the muscle to acetylcholine, slowing the rate of contraction and almost stopping relaxation.

Extracts prepared in a similar way from *Gymnodinium vitiligo* Ballantine (1956), which is non-toxic to fish, were used on both hearts and muscles, and had no action at all. Thus it is assumed that any pharmacological reactions are due to the toxin and not to other cell products with which the extracts are undoubtedly contaminated.

Frog sartorius nerve-muscle preparation

Fingerman *et al*. (1953) have shown that paralytic shellfish poison extracted from digestive glands of poisonous shellfish is a neuromuscular toxin. They found that it eliminates the transmission of an impulse across the frog nerve-muscle junction, and conclude that the action of this toxin is curare-like. We have carried out similar experiments using neutral extracts of *G. veneficum*, and the preparation becomes inexcitable to stimulation through the nerve, without the nerve itself being affected. End-plate potentials can be recorded just after indirect excitability has disappeared, but these also decrease. The muscle does not respond to direct stimulation when the end-plate potential drops. In contrast to the conclusions of Fingerman *et al*. we find, as shown below (p. 182), that the membrane potential in the muscle has been destroyed and therefore the action of this toxin differs from that of curare. The 'acid extract' also renders the muscle inexcitable, but we have not followed this up.

Mechanical response of muscles.

The time course of the effect of the toxin on the mechanical response of frog and *Mytilus* muscles (Figs. 3, 4) shows that as the excitability decreases the rates of rise and fall of tension become slower.

The frog sartorius nerve-muscle preparation was mounted in a muscle bath and connected to an isometric lever. Tensions were recorded photoelectrically and displayed on a cathode-ray tube (Hill, 1949*a*). Stimulation could be applied to either nerve or muscle. When the anterior byssus retractor muscle of *Mytilus* was used the muscle was mounted on a multi-electrode assembly and again connected to the same recording apparatus (Abbott & Ritchie, 1951). Stimulation was by square wave pulses from an electronic stimulator.

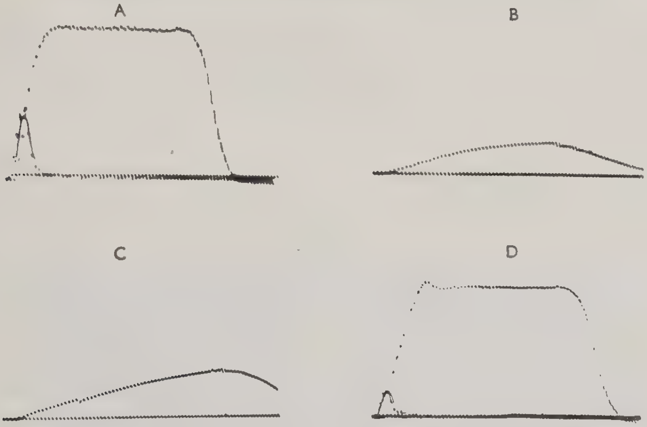

Fig. 3. Mechanical response of frog sartorius nerve-muscle preparation. Twitch and tetanic (25/sec) stimulation applied in each figure. Time base 1·5 sec, temp. 15° C. A, indirect maximal stimulation of untreated preparation; B, indirect stimulation after 10 min soak in neutral toxin extract; the twitch has disappeared; C, direct (muscle) stimulation immediately after B; D, indirect stimulation after 30 min soak out in normal Ringer.

Figs. 3, B, C show that even when the active isometric tension has been reduced to one third by the toxin (10 min immersion), the response by frog sartorius muscle to direct stimulation is identical with that to indirect stimulation, a fact not consistent with the action of a curare-like substance. It can also be seen that the twitch has by this time disappeared and that tetanic tension rises and falls very slowly. After 5 min soak-out in normal Ringer the tetanus tension had recovered to about 50% and was completely restored (Fig. 3D) in 30 min, together with the twitch.

Mytilus muscle (Fig. 4) similarly becomes inexcitable in the presence of the toxin. In a toxin-treated muscle successive tetani show facilitation (B, C, D) when stimulated by a voltage which gives a maximal response in the untreated muscle (A). Increased voltage, however, gives a tetanus equivalent to that shown at the end of a series of facilitating tetani (D). Thus the voltage required to give a maximal response steadily increases as the toxin acts.

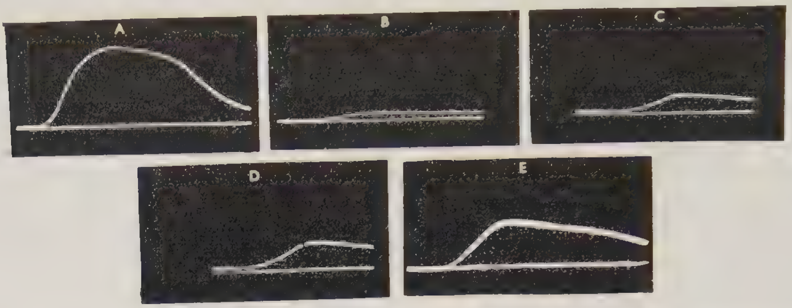

Fig. 4. Isometric contractions of *Mytilus* anterior byssus retractor muscle. Tetanus duration 5 sec, frequency 4/sec, stimulus strength 9 V, width 3 msec, temp. 15° C. A, untreated muscle; B–D, after 50 min soak in *Gymnodinium* culture, a series of tetani was given at 30 sec intervals, of which B is the first tetanus, C is the third and D is the sixth tetanus; E, tetanus after 3 h soak out in sea water.

Action potential measurements

From our experiments on intact animals, where death occurs before the muscles become inexcitable to direct stimulation, it is certain that the toxin must be acting somewhere on the nervous system. Since the experiments on the frog sartorius nerve-muscle preparation showed that the muscle response disappeared long before the end-plate potentials and nerve action potentials, it was necessary to investigate the action of the toxin on non-myelinated nerves, nerves with sheaths removed, and ganglia. Kellaway (1935) showed that sensory nerve endings in frog skin were quickly blocked by paralytic shellfish poison.

Lengths of nerve about 4 cm were dissected and mounted between forceps in an arrangement similar to that of Keynes & Lewis (1951), with the ends of the nerve higher than the central portion which was immersed in the bathing solution all the time. The whole assembly was on a Palmer stand so that the whole nerve could be immersed between records. A recorded action potential means that the whole immersed length of the nerve is conducting. Nerves were first soaked in their normal bathing solution and excitability checked. Toxin was then added and the effect with time noted.

Leg nerve of *Maia* (spider crab) was used as an example of a non-myelinated nerve with very little sheath (Fig. 5). The bathing solution was that described by Welsh (1936). Within 3 min of immersion in toxin the action potential had become spread out into a series of humps (B) and then steadily decreased in amplitude over about 45 min (C, D). Washing in normal Ringer restored the action potential.

When either ray wing motor nerve or frog sciatic nerve was tested the toxin was found to have only a small, slow effect on the intact nerve. When the nerve sheath is removed the nerves remain viable in normal Ringers (ray Ringer— Babkin, Bowie & Nicholls, 1933; frog Ringer— Hill, 1949*b*) for many hours, but conduction is quickly blocked in the presence of the toxin (less than 30 min). The inability of the toxin to block sheathed nerves is probably due to the fact that the molecule, which we believe to be large (p. 171), cannot penetrate the sheath.

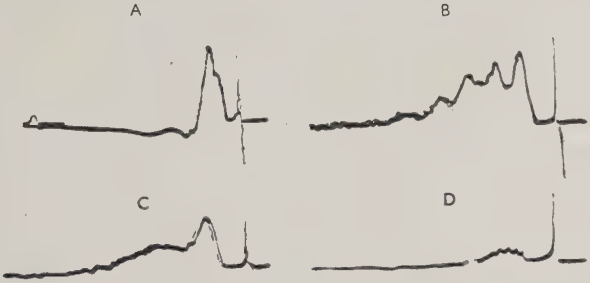

Fig. 5. Action potentials in *Maia* leg nerve. Time base sweep duration 150 msec. (right to left), temp. 15° C. A, untreated nerve; B, after 3 min in *Maia* Ringer with *Gymnodinium* toxin extract added; C, after 10 min in toxin; D, after 45 min in toxin.

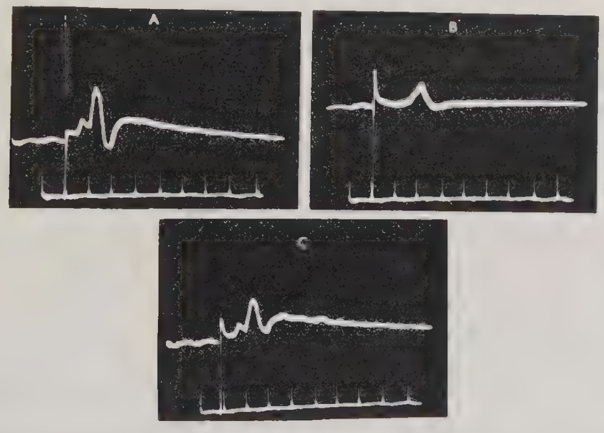

Fig. 6. Ganglionic potentials in isolated rat superior cervical ganglion. Time markers 50/sec, temp. 37° C. A, untreated ganglion; B, after 10 min in toxin; C, after 15 min soak out in normal Kreb's solution.

Ganglia

Preganglionic, ganglionic and postganglionic action potentials of isolated superior cervical ganglia of rats (at 37° C) were recorded for us by Dr J. P. Quilliam. Addition of toxin to the bathing solution slowly depressed the preganglionic potentials and more quickly depressed the postganglionic potentials, both without appreciable change in their time course. The ganglionic potentials (Fig. 6), on the other hand, were depressed and eventually blocked, and their characteristics altered. In the untreated state there is an after-potential lasting nearly $\frac{1}{5}$ sec (A). As the toxin acts this after-potential is destroyed quicker than the spike (B). The potential returns to normal on soak-out of the toxin (C).

Membrane potentials

The disappearance of action potentials of nerves, and of both action potentials and end-plate potentials of muscles, led us to measure the effect of the toxin on membrane potentials. Micro-electrodes of the type described by Graham & Gerard (1946) and Ling (1948) were inserted into muscle fibres of *Maia* leg, dogfish jaw muscle and frog sartorius. The membrane potential value was checked in the untreated muscle and its change noted when toxin was added. In *Maia* muscle the potential dropped over a period of 1–2 h from an initial value of 65 mV to less than 10 mV and the fibres became inexcitable. The effect on frog and dogfish muscle was more rapid, potentials falling over the same range within 30 min. Thus, apparently, although the precise point at which the toxin acts to kill an animal is unknown, it functions by depolarising excitable membranes.

Frog skin

Further evidence that the toxin reduces cellular potentials was obtained with frog skin. Isolated pieces of frog skin taken from the abdominal surface were mounted in an apparatus similar to that described by Koefoed-Johnsen, Ussing & Zerahn (1952). The skin separated two chambers, each containing Ringer, and the potential difference across the skin was measured (Fig. 7). The skin potential dropped within 10 min from about 70 mV positive on the inside to a reversed potential of about 10 mV negative on the inside, when toxin is added to the Ringer on the inside of the skin (curve *a*). When the toxin was added to the Ringer outside the skin, the potential dropped much more slowly and to only about half the original value (curve *b*).

The experiments were repeated with scallop liver extract added to the inside Ringer (2 mg extract ≡ 2 mouse units to 4 ml. Ringer). The potential again fell slowly and to about half the original value (curve *c*).

In all experiments the potential was restored to its original level within a few minutes of replacing the toxin with normal Ringer.

Ussing & Zerahn (1951) have explained the standing potential across frog skin in terms of an equivalent electrical circuit in which the sodium pump provides a driving potential in series with a resistance to the movement of sodium ions, with a passive resistance to other ions acting in parallel to this circuit. Disappearance of the potential could occur from a blockage of the sodium pump or from a greatly decreased passive skin resistance. A measure of the skin resistance was obtained by forcing extra current through the skin from an outside source. The potential recorded across the skin varies with the current passed in a nearly linear manner and the slope of the potential–current relationship represents the effective total conductance of the skin. It was found that the skin conductance, and therefore the resistance, did not alter measurably when the skin potential was abolished by the toxin. It therefore appears that the toxin is acting on the sodium exchange mechanism. The brief description given recently by Koefoed-Johnsen & Ussing (1956) of a modified theory of the origin of frog skin potential is discussed later (p. 186).

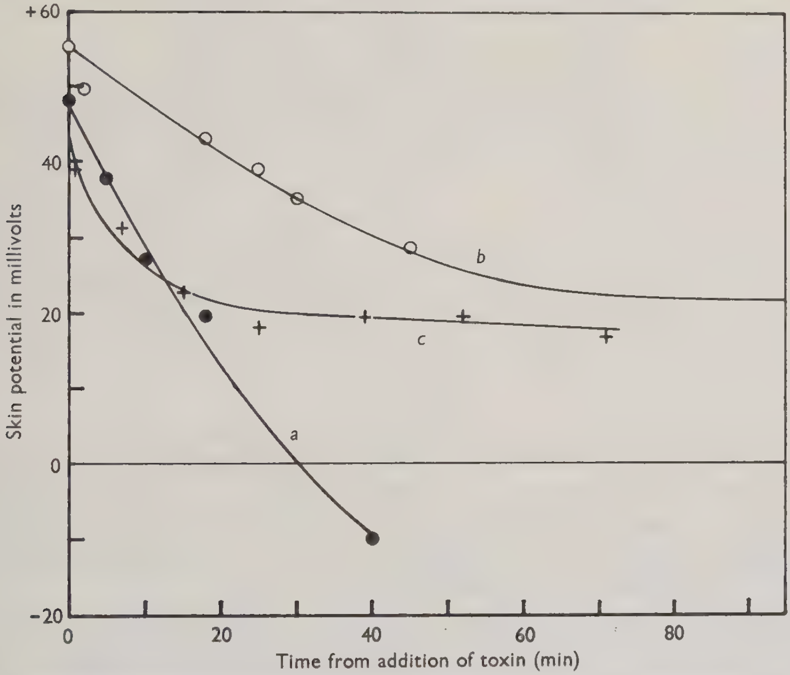

Fig. 7. Time course of decay of potential across isolated frog skin in the presence of toxins. Ringer on both sides of skin, temp. 15° C. Curve (*a*), neutral *Gymnodinium* toxin extract added to inside surface of skin; curve (*b*), neutral *Gymnodinium* toxin added to outside surface of skin; curve (*c*), scallop liver extract added to inside surface of skin.

Other experiments

Two other lines of approach have been made and preliminary results are quoted here, though further investigation is proceding.

The influence of the toxin on the rate of oxygen consumption by frog and *Mytilus* muscles has been measured, and we find that the rate is increased by 58 and 28 % respectively (Table 2).

It is obvious that a great deal of information can be obtained from studying, with radioactive potassium and sodium, the influence of the *Gymnodinium* toxin on the ionic exchanges across cell membranes. In resting nerve and muscle the membrane potential is closely related to the ratio of potassium concentrations on the two sides of the membrane. The speed with which the toxin acts, and also the rate of recovery, is surprisingly fast if the entire potential decrease is due to leakage of potassium out of the cell.

TABLE 2. RESTING RESPIRATION RATES OF FROG AND *MYTILUS* MUSCLE, AT 19·7° C

(μl O_2/mg wet wt/h)

	Normal Ringer	Toxin-treated Ringer
Frog	0·165	0·261
Mytilus	0·042	0·054

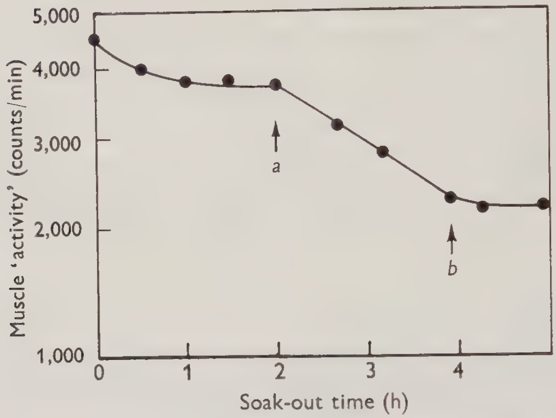

Fig. 8. Effect of *Gymnodinium* toxin on outward flux of potassium from frog sartorius muscle. Muscle soaked overnight at 0° C in Ringer labelled with ^{42}K. Toxin added at time *a*, normal Ringer restored at time *b*, soak-out temp. 16° C.

Frog sartorius muscle was used for exchange experiments with radioactive ^{42}K. The muscle was soaked overnight at 0° C in labelled Ringer. Next morning it was transferred to normal Ringer at room temperature to wash out the radioactivity. The activity of the muscle was counted at intervals as soaking out continued. When the extracellular potassium had diffused away, the activity (after correcting for decay of the isotope) decreased exponentially with time over several hours. The slope of the curve gives a measure of the potassium efflux, and the half-time of exchange (Fig. 8) is about 30 h. Toxin was then added to the soak solution, at the time indicated on the curve. The muscle was inexcitable within 20 min. The efflux rate of potassium increased greatly but the time for half exchange was about 3 h, so that the membrane potential disappeared long before the internal potassium level had fallen to the requisite value to account for the depolarization.

The experiments described in this section are summarized in Table 3.

TABLE 3. SUMMARY OF THE ACTION OF *GYMNODINIUM* TOXIN ON VARIOUS TISSUES

Preparation	Effect of 'Neutral' toxin extract	Page reference
Buccinum heart	Arrest in systole	177
Frog rectus abdominus	No contracture; reduction of sensitivity to acetylcholine: slowing of relaxation	178
Frog sartorius nerve-muscle	(1) indirect stimulation: muscle inexcitable, nerve slightly affected, end-plate potentials decrease and disappear. Muscle twitch quickly goes, tetanic tension decreases and becomes slower in rise and fall	178
	(2) direct stimulation: mechanical response decreases and is always identical with response to indirect stimulation	179
	Muscle respiration rate increased	183
	Efflux rate of potassium greatly increased	184
Mytilus anterior byssus retractor	Becomes inexcitable. Progressive threshold increase, mechanical response is slowed	179
Nerve, crab	Action potential spreads out into several humps, and disappears	180
Nerve, de-sheathed frog sciatic	Blocked	181
Superior cervical ganglion of rat	Ganglion potential depressed and then blocked. After-potential disappears first	181
Frog skin	Toxin on inside, potential decreases and reverses in sign. No measurable change in skin resistance. Toxin on outside, very slow partial drop in potential	182

DISCUSSION

Sommer & Meyer (1937) have suggested that the toxin produced by dinoflagellates and causing paralytic shellfish poisoning may be different from that liberated by other dinoflagellates associated with mass mortality of fish. Our observations on the toxin from *Gymnodinium veneficum* tend to confirm this view, as we are unable to render shellfish poisonous with it, and it differs from the poison in lyophilized scallop liver extract, both in action on fish (p. 176) and mice (p. 176) and on isolated frog skin (p. 182). Whereas Fingerman *et al.* have indicated that the paralytic shellfish poison operates in a curare-like manner, we have shown that the *Gymnodinium* toxin depolarizes the excitable membrane.

Our evidence from the frog skin, considered in the light of Ussing's original hypothesis of the origin of the skin potential, suggests that the toxin blocks the sodium pump—particularly as the skin resistance is not measurably altered. In the case of nerve and muscle, however, blockage of the sodium pump could only result in depolarization of the membrane after sufficient potassium had leaked out, in exchange for the sodium entering the fibre—a process which would take very much longer than is needed for depolarization with the toxin. In a similar way the recovery is much too rapid to be explained by a reversal of this process. Moreover, analysis of muscles which have been rendered completely inexcitable by the toxin shows that they still contain considerable amounts of potassium. The tracer experiments mentioned above (p. 184) also show that although the potassium efflux increases in the presence of the toxin, the exchange is not rapid enough to explain the depolarization (in terms of a concentration cell).

Koefoed-Johnsen & Ussing (1956) have recently modified their hypothesis and now suggest that two separate membranes are concerned with the potential in frog skin: on the outer surface there is a membrane selectively permeable to sodium, and on the inner surface a membrane which is selectively permeable to potassium. A sodium-potassium exchange pump, located in the inner membrane, maintains a low sodium and high potassium concentration in the skin cells. The potential measured across the skin is the sum of the sodium diffusion potential across the outer membrane and the potassium diffusion potential across the inner membrane. Such a potential would decrease if the inner membrane became permeable to sodium ions; and if the permeability became greater than that of the outside membrane the potential could certainly temporarily reverse. We find that the toxin produces a reversal of potential. Certainly the selectivity of the toxin for the inside of the skin indicates that its site of action is there.

An increase in sodium permeability would also reduce the membrane potential in nerve and muscle cells. Such a permeability increase occurs during the rising phase of an action potential, when the potential drops rapidly and reverses in sign.

From these arguments we conclude that the *Gymnodinium* toxin is acting on the nervous system of animals by depolarizing excitable membranes. We feel that this probably occurs by interference with the sodium exchange mechanism. The speed of action of the toxin makes it improbable that it is only stopping sodium extrusion, and the more probable explanation is that it allows rapid entry of sodium into the cells.*

SUMMARY

Methods of extraction of the toxin from cultures of *Gymnodinium veneficum* are described. This is now done by dialysis and evaporation under reduced pressure. The toxin molecule must be large, as it cannot penetrate a dialysis membrane; it is soluble in water and the lower alcohols, but insoluble in ether and chloroform. It is unstable in acids, turning into another toxic product, and is decomposed by hot alkali, though in neutral solution is more or less thermostable. The toxin as it occurs in sea water is not the same as paralytic shellfish poison, but there are some resemblances between this and the 'acid extract', though much more work is needed to check this point.

The action of the toxin on a variety of animals is described, and an attempt

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* See note at end of paper.

is made to devise an approximate assay technique using gobies as the test animals.

The action has also been observed on a range of isolated preparations, and although the final conclusion as to the mode of action is not quite clear, we feel confident that the site of action in whole animals is in the nervous system, probably acting on ganglion transmission. With regard to the mode of action it depolarizes nerve and muscle membranes. It also abolishes the potential across frog skin without measurably altering the skin resistance.

This depolarization probably occurs by interference with the sodium exchange mechanism, allowing rapid entry of sodium into the cells.

Note added in proof

Preliminary experiments have been carried out on the effect of this toxin on sodium permeability of frog sartorius muscle using the radioisotope ^{22}Na. Pairs of sartorius muscles were isolated. From each pair, one muscle was soaked in normal frog Ringer labelled with ^{22}Na and the other soaked in similar labelled Ringer to which toxin was added in sufficient quantity to render the muscle inexcitable. The exchange of sodium across the muscles was followed in terms of the radioactivity of the muscles: the muscles were removed at intervals, surface liquid blotted off and activity measured. Activity (and total sodium) of the toxin treated muscles increased more rapidly and to a higher final level than that of the untreated muscles. The tissues were later soaked in non-radioactive Ringer solutions and the decrease in activity followed. The activity of the normal muscle decreased rapidly at first corresponding to exchange in the extracellular sodium, and then more slowly as intracellular sodium exchanged. In the case of the toxin-treated muscle the exchange was very rapid and large, and consisted only of the one fast phase. It appeared that the membrane had become so permeable to sodium that the exchange from inside the cells had become similar in time course to that of the extracellular sodium.

The conclusions of this paper are of necessity preliminary and need verifying with a purified sample of the toxin. This, however, must await further development of the technique of producing large quantities of material and subsequent purification.

REFERENCES

ABBOTT, B. C. & RITCHIE, J. M., 1951. The onset of shortening in striated muscle. *J. Physiol.*, Vol. 113, pp. 336–45.

BABKIN, B. P., BOWIE, D. J. & NICHOLLS, J. V. V., 1933. Structure and reactions to stimuli of arteries (and conus) in the elasmobranch genus *Raja*. *Contr. Canad. Biol.*, Vol. 8, pp. 207–25.

BAINBRIDGE, R., 1953. Studies on the interrelationships of zooplankton and phytoplankton. *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 385–447.

Ballantine, Dorothy, 1956. Two new marine species of *Gymnodinium* isolated from the Plymouth area. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 467–74.

Ballantine, Dorothy & Morton, J. E., 1956. Filtering, feeding and digestion in the lamellibranch *Lasaea rubra*. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 241–74.

Brongersma-Sanders, M., 1948. The importance of upwelling water to vertebrate paleontology and oil geology. *Verh. Akad. Wet. Amst., Afd. natuurkunde*, Sect. 2, Deel 45, No. 4, 112 pp.

Davis, C. C., 1948. *Gymnodinium brevis* n.sp., a cause of discolored water and animal mortality in the Gulf of Mexico. *Bot. Gaz.*, Vol. 109, pp. 358–60. (Contrib. no. 17, Univ. Miami Mar. Lab.)

Fingerman, M., Forester, R. H. & Stover, J. H., 1953. Action of shellfish poison on peripheral nerve and skeletal muscle. *Proc. Soc. exp. Biol., N.Y.*, Vol. 84, pp. 643–6.

Graham, J. & Gerard, R. W., 1946. Membrane potentials and excitation of impaled single muscle fibres. *J. cell. comp. Physiol.*, Vol. 19, pp. 135–44.

Hashimoto, Y. & Migita, M., 1952. On the shellfish poisons. 1. Inadequacy of acidulated alcohols with HCl as solvent. *Bull. Tokai Fish. Res. Lab.*, Vol. 3B, pp. 77–84.

Hayes, Helen L. & Austen, T. S., 1951. The distribution of discolored sea water. *Tex. J. Sci.*, Vol. 3, pp. 530–41.

Hill, A. V., 1949*a*. Myothermic methods. *Proc. roy. Soc.* B, Vol. 136, pp. 228–41.

—— 1949*b*. Is relaxation an active process? *Proc. roy. Soc.* B, Vol. 136, pp. 420–35.

Ingle, R. M., 1954. Irritant gases associated with Red Tide. *Univ. Miami mar. Lab., Special Service Bull.*, No. 9. (Florida State Bd of Conservation.)

Kellaway, C. H., 1935. The action of mussel poison on the nervous system. *Aust. J. exp. Biol. med. Sci.*, Vol. 13, pp. 79–94.

Keynes, R. D. & Lewis, P. R., 1951. The resting exchange of radioactive potassium in crab nerve. *J. Physiol.*, Vol. 113, pp. 73–98.

Koch, H. J., 1939. La cause des empoissonements paralytiques provoqué par les moules. *C.R. Ass. Franc. Avanc. Sci., Sean. Sess.* 63, *Liége*, pp. 654–7.

Koefoed-Johnsen, V. & Ussing, H. H., 1956. Nature of the frog skin potential. *XX Congr. int. Physiol.*, pp. 511–512.

Koefoed-Johnsen, V., Ussing, H. H. & Zerahn, K., 1952. The origin of the short-circuit current in the adrenaline stimulated frog skin. *Acta physiol. scand.*, Bd. 27, pp. 38–48.

Ling, G., 1948. Effect of stretch on membrane potential in frog muscle. *Fed. Proc.* Part 1, vol. 7, p. 72.

Marshall, S. M. & Orr, A. P., 1955. On the biology of *Calanus finmarchicus*. VIII. Food uptake, assimilation and excretion in adult and stage V *Calanus*. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 495–529.

Medcof, J. C., Leim, A. H., Needler, A. R., Needler, A. W. H., Gibbard, J. & Naubert, J., 1947. Paralytic shellfish poisoning on the Canadian Atlantic coast. *Bull. Fish. Res. Bd Can.*, Vol. 75, pp. 1–32.

Müller, H., 1935. The chemistry and toxicity of mussel poison. *J. Pharmacol.*, Vol. 53, pp. 67–89.

Needler, A. B., 1949. Paralytic shellfish poisoning and *Goniaulax tamarensis*. *J. Fish. Res. Bd Can.*, Vol. 7, pp. 490–504.

Nightingale, H. W., 1936. *Red Water Organisms: Their Occurrence and Influence upon Marine Aquatic Animals, with Special Reference to Shellfish in Waters of the Pacific Coast*, 24 pp. Seattle, Washington: Argus Press.

Prinzmetal, M., Sommer, H. & Leake, C. D., 1932. The pharmacological action of 'mussel poison'. *J. Pharmacol.*, Vol. 46, pp. 63–75.

RIEGEL, B., STANGER, D. W., WILKHOLM, D. M., MOLD, J. D. & SOMMER, H., 1949*a*. Paralytic shellfish poison. IV. Bases accompanying the poison. *J. biol. Chem.*, Vol. 177, pp. 1–6.

—— 1949*b*. Paralytic shellfish poison. V. The primary source of the poison, the marine plankton organism, *Gonyaulax catenella*. *J. biol. Chem.*, Vol. 177, pp. 7–11.

SOMMER, H. & MEYER, K. F., 1937. Paralytic shellfish poisoning. *Arch. Path.* (*Lab. Med.*), Vol. 24, pp. 560–98.

SOMMER, H., MONNIER, R. P., RIEGEL, B., STANGER, D. W., MOLD, J., WIKHOLM, D. M. & KIRALIS, E. S., 1948. Paralytic shellfish poison. I. Occurrence and concentration by ion exchange. *J. Amer. chem. Soc.*, Vol. 70, pp. 1015–18.

SOMMER, H., RIEGEL, B., STANGER, D. W., MOLD, J. D., WILKHOLM, D. M. & MCCAUGHEY, MARGARET B., 1948. Paralytic shellfish poison. II. Purification by chromatography. *J. Amer. chem. Soc.*, Vol. 70, pp. 1019–21.

SOMMER, H., WHEDON, W. F., KOFOID, C. A. & STOHLER, R., 1937. The relation of paralytic shellfish poison to certain plankton organisms of the genus Gonyaulax. *Arch. Path.* (*Lab. Med.*), Vol. 24, pp. 537–59.

STEPHENSON, N. R., EDWARDS, H. I., MACDONALD, B. F. & PUGSLEY, L. I., 1955. Biological Assay of the toxin from shellfish. *Canad. J. Biochem. Physiol.*, Vol. 33, pp. 849–57.

USSING, H. H. & ZERAHN, K., 1951. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta physiol. scand.*, Bd. 23, pp. 110–27.

WELSH, J. H., 1936. Chemical mediation in crustaceans. I. The occurrence of acetylcholine in nervous tissues and its action on the decapod heart. *J. exp. Biol.*, Vol. 16, pp. 198–219.

WOODCOCK, A. H., 1948. Note concerning human respiratory irritation associated with concentrations of plankton and mass mortality of marine organisms. *J. mar. Res.*, Vol. 7, pp. 56–62.

ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

A NEW MUSCLE PREPARATION FOR THE STUDY OF OPTICAL CHANGES DURING CONTRACTION

By B. C. Abbott and J. Lowy

Nature, Lond., 1956, Vol. 177, pp. 788–9

Pieces cut from the mantle muscle of squid have been used to study transient optical changes in the muscle when stimulated. When alive and healthy this muscle is very transparent. Two types of changes are described. The first is a small increase in transparency which begins during the falling phase of the action potential. At room temperature the peak of this increase is at 22 msec after the stimulus. The first sign of tension increase occurs at 19 msec, with no evidence of a preceding latency relaxation.

This transparency increase is followed by a large opacity increase due to light scattering. The scattering closely follows the time course of the twitch, and about 30% of the light is scattered at the twitch peak. B.C.A.

RESTING TENSION IN SNAIL MUSCLE

By B. C. Abbott and J. Lowy

Nature, Lond., 1956, Vol. 178, pp. 147–8

The retractor pharynx of the snail extends to great lengths, even under a small load and its ability to exert a resting tension has been doubted.

The experiments described demonstrate that the muscle has a very wide range of working length. The isometric tensions produced during tetanus have been measured at various lengths, and it is demonstrated that although appreciable tension is produced at as little as 12 mm, the maximum active tension occurs at about 40 mm. Above this length active tension decreases again: as in vertebrate striated muscle.

At lengths above that for maximum active tension, the resting muscle exerts a true resting tension and tears with a breaking stress of about 1·5 kg/cm^2. B.C.A.

CILIARY FEEDING MECHANISMS OF BRACHIOPODS

By D. Atkins

Nature, Lond., 1956, Vol. 177, pp. 706–7

A preliminary account is given of the ciliary feeding mechanisms of brachiopods based on the articulates *Terebratulina*, *Gryphus* and *Macandrevia* and the inarticulate *Crania*. In all these, the lateral cilia, beating across the length of the filaments and from the inside to the outside of the lophophoral spaces, create the main water current, which in adults, whether with plectolophus or with spirolophus lophophores, enters laterally and leaves the shell anteriorly. In young stages with trocholophus and with early schizolophus lophophores, the inhalant current is single and median, setting into the bell-shaped lophophore, while the exhalant current escapes between the filaments all around. As the anterior indentation increases with age, two lateral arms being formed, the median inhalant current becomes divided into two.

The frontal currents in all the species examined are capable of reversal. Small particles are conveyed by the frontal cilia toward the bases of the filaments and eventually reach the mouth; large particles and collections of particles are conveyed to the tips of the filaments and are finally rejected from the shell. In a thick culture of *Chlorella*, etc., the frontal cilia beat toward the tips of the filaments. This reversal of the frontal currents of the filaments appears to be due to reversal of the ciliary beat and not to adjacent tracts of cilia beating in opposite directions as in certain bivalves.

In *Terebratulina retusa*, *Gryphus vitreus* and *Macandrevia cranium*, abfrontal cilia are present on the filaments and these beat continuously toward their tips. D.A.

OBSERVATIONS ON LUMINESCENCE IN SEA PENS (PENNATULACEA)

By D. Davenport and J. A. C. Nicol

Proc. roy. Soc., B, 1956, Vol. 144, pp. 480–96

Luminescent responses of a sea pen, *Leioptilus gurneyi*, obtained off the Pacific coast of the United States, proved favourable for physiological studies. Light is emitted by autozooids and siphonozooids. Mechanical and electrical stimuli evoke luminescent waves which pass over the rachis at a velocity of 26 cm/sec (20° C). Consecutive flashes increase in intensity owing to neuroeffector facilitation. A flash has a latent period of 0·18 sec (total duration of 1–2 sec) and reaches maximal intensity in 0·2 sec from first deflexion. Strong mechanical and prolonged electrical stimulation produce a refractory state in which transmission of luminescent waves ceases. Slow progressive spread of luminescence can then be induced by maintained repetitive stimulation. This effect is ascribed to internuncial facilitation overcoming fatigue in the nerve net. J.A.C.N.

SYSTEMATICS OF THE MARINE-BUG

By Dennis Leston

Nature, Lond., 1956, Vol. 176, pp. 427–8

Study of male and female genitalia, head and mouthparts, abdomen, dorsal glands and other structures shows that the bug, *Aepophilus bonnairei*, hitherto regarded as comprising a monotypic family of uncertain affinities, is a saldid. The lateral abdominal 'strigil' of males confirms this view. Other saldid species from widely separated genera show a tendency to live a submarine life, but *A. bonnairei* has progressed furthest along this path and has well-defined structural and behavioural modifications suiting it to life at the lowest *Fucus* zone. It can survive submergence for at least 49 h in sea water. D.L.

PRESSURE RECEPTORS IN THE FINS OF THE DOGFISH *SCYLLIORHINUS CANICULA*

By Otto Lowenstein

J. exp. Biol., 1956, Vol. 33, pp. 417–21

The presence of so-called terminal corpuscles in the connective tissues of the fins of *Scylliorhinus canicula*, first described by Wunderer in 1908, is confirmed.

It is demonstrated that they are pressure receptors with a slow rate of adaptation.

From their topographic distribution and from their mode of response to mechanical stimulation, it is postulated that the terminal corpuscles serve as proprioceptors in the widest sense of the term by signalling the spatio-temporal patterns of active or passive deformation of the fin.

Their topographic distribution makes it possible to distinguish their responses from those of sense endings associated with the muscles or tendons of the fin. O.L.

NERVOUS REGULATION OF LUMINESCENCE IN THE SEA PANSY *RENILLA KÖLLIKERI*

By J. A. C. Nicol

J. exp. Biol., 1955, Vol. 32, pp. 619–35

During a visit to the Scripps Institution of Oceanography, advantage was taken of the opportunity to study luminescence in the sea pansy *Renilla köllikeri*. When stimulated, *Renilla* produces luminescent waves, which are under control of a non-polarized nerve net. The response is subject to facilitation, which occurs terminally, at the neuro-photocyte junctions. Facilitation is analysed in detail, and a facilitation-decay curve presented. Between successive bursts of stimuli, facilitation may persist for some 10 min. Measured response-parameters were as follows: latent period, 0·12 sec; local duration of response, 1 sec; time to reach maximal intensity, 0·22 sec; conduction speed of luminescent wave, 9 cm/sec (16–17° C). The refractory period of the response is about 0·2 sec, and increases under repetitive stimulation. When strongly excited the animal passes into a hyper-excitatory state, in which luminescent waves continue to arise long after stimulation has ceased.

J.A.C.N.

OBSERVATIONS ON THE ANATOMY AND MODE OF LIFE OF *LASAEA RUBRA* (MONTAGU) AND *TURTONIA MINUTA* (FABRICIUS)

By E. Oldfield

Proc. malacol. Soc. Lond., 1955, Vol. 31, pp. 226–49

The anatomy of *Lasaea rubra* and *Turtonia minuta* is described. Certain organs such as the gills and stomach show simplicity of structure which has probably been acquired secondarily in relation to small size.

In both genera the mode of reproduction is specialized for protection of the developing embryos and consequently relatively few eggs are produced, but these are large and heavily laden with yolk. *Lasaea* and *Turtonia* show entirely different methods for protecting the developing young; in *Lasaea* the embryos are incubated within the suprabranchial chamber, whereas in *Turtonia* the embroys develop within a gelatinous capsule which is attached to the byssus of the female. In both genera the free veliger stage is completely suppressed; a study of the embryology is in progress.

The systematic position of the two genera is discussed and it appears that *Turtonia* should not be included together with *Lasaea* and *Kellia* in the family Erycinidae.

E.O.

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ON SOME COPEPODA FROM PLYMOUTH, MAINLY ASSOCIATED WITH INVERTEBRATES, INCLUDING THREE NEW SPECIES

By R. U. Gooding
From the Plymouth Laboratory[1]

(Text-figs. 1–6)

Work on copepods at the Plymouth Marine Laboratory in 1954–55 revealed some new species and some which have been described but whose occurrence in that region had not previously been reported. Specimens from both these categories are discussed in the present paper.

The names of copepods associated with the fish of the region are comparatively well known through the work of Bassett-Smith, Leigh-Sharpe and others (although the present record for *Peniculus fistula* suggests that the list is still incomplete; and others might bear re-investigation); but little attention has been paid to invertebrates as hosts. Norman & Scott (1906) and Leigh-Sharpe's paper on the Herpyllobiids (1926) together formed the basis of the 1931 Plymouth Fauna list for records of species associated with these animals; and additions have later been made in the following papers: Gray (1933), Gurney (1933), Atkins (1934), and Leigh-Sharpe (1933). These references, in conjunction with the present publication, indicate that the invertebrate fauna of Plymouth is probably as prolific a source of associated copepods as any.

My sincere thanks are due to Dr J. P. Harding, under whom this work was done, and to Dr Paul Illg in particular for reading this paper and for giving me the benefit of so detailed and constructive a criticism; to the Director for facilities at the Marine Laboratory, Plymouth, and for constant encouragement; to the members of the Staffs of that Laboratory and this Department for help, host specimens and advice at the times I needed them most; and, finally, to the Government of Barbados, B.W.I., for allowing me to apply funds from a 1950 Scholarship to this study.

DEFINITIONS

The word 'associated' has been used in the title and throughout this paper because it is felt that terms like 'parasitic', 'semi-parasitic', 'commensal', etc., should be restricted to cases where there is some definite evidence about the nature of the association, and little work to this end has been done on the Copepoda (cf. especially Baer, 1951; Gotto, 1954). Symbiosis, in its widest sense (Allee, Emerson, Park, Park & Schmidt, 1949; Davenport, 1955; etc.), is perhaps broad enough for use in this context, but the term adopted has the advantage of simplicity and is unlikely to cause confusion through previous usage in a stricter sense. There seems no need for formal definition except to say that, in the Copepoda, it is intended to include the range from forms firmly attached to a single host, usually of a particular species, to those with as free-and-easy a host requirement as some lichomolgids; and may be applied equally to the earlier instars, if they are found in contact with the host, as to adults. But it still seems necessary to distinguish between the partners in the association: I suggest that the concept of 'host' is already wide enough, and that 'associate' should here refer to the copepod.

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Several terms have from time to time been used to differentiate the regions of the copepod body, some purely descriptive, others borrowed to express opinions on comparative crustacean morphology. I am adopting, for descriptive work, those introduced by Sars (1901), but, in addition, have added 'cephalothorax'—for use in a restricted sense—and proposed 'prosome'—as antithetic to 'urosome'—to include both 'cephalosome' and 'metasome'. (There seem definite advantages in retaining Sars's original meanings for these terms in preference to Wilson's, 1932, p. 9, interpretation.) These regions may now be defined:

Prosome: the anterior region of the body, commonly limited posteriorly by a major articulation;
Cephalosome: the head region when this includes only the somite of the maxillipedes;
Cephalothorax: the head region including, in addition to the maxillipedal somite, pedigerous segments in a fused complex;
Metasome: those free thoracic segments in front of the major articulation;
Urosome: that part of the body behind the major articulation.

This terminology is applicable to all divisions of the Copepoda despite the fact that, for instance, in the Calanoida, the urosome may include only the genital and succeeding segments, while in some Caligoida it contains all segments behind the third pedigerous; but it is hoped that the division of the body into two main parts will facilitate description especially in the Cyclopoida, where the anterior region is usually sharply defined from the posterior by a considerable difference in width.

The homology of the poecilostome mouthparts has also been a source of some discussion: whether the same number as is present in other groups may here be distinguished or, if not, which is lacking. Lang (1946*a*) has discussed the arguments but, despite his conclusion—partly modified in 1948

(p. 24)—I shall presume that a mandible, maxillule, maxilla, and maxilliped can be identified in the two new poecilostomes described here. Also, although it now seems generally agreed that the somite bearing the maxillipedes is the first thoracic segment (permanently included in the head region), I have tried to avoid confusion by using the terms 'pedigerous' instead of 'thoracic', when discussing the somites bearing the thoracic legs as a whole, or that of the fifth leg by itself, and 'prosomal' to refer to those bearing legs one to four. In the descriptions of many appendages, 'articulated process' denotes both setae and spines (for a summary of the differences between these see Gurney, 1931, p. 38), 'process' being restricted to the usual carcinological connotation of an unarticulated projection.

The following is a list of abbreviations which have been used in referring to the swimming legs:

P1, P2, etc.—first, second, etc., pairs;
basp.—basipodal segments;
(basp. 1—coxa; basp. 2—basipodite);
end.—inner ramus (endopodite);
exp.—outer ramus (exopodite);
Si—inner border of segments;
St—terminal border of segments;
Se—outer border of segments.

I have followed Sewell (1949) in differentiating the spines by Roman, the setae by Arabic numerals.

METHODS

Collection methods are dealt with under the individual records.

Much of the examination, and all measurements and drawings (for which a camera lucida was used) were made from preparations cleared and mounted temporarily, in lactic acid. (I am very grateful to Dr Harding for suggesting this method.) Whole specimens were mounted in a 'hanging drop' to avoid compression by the cover-slip. For measuring, the specimens were oriented dorsal surface uppermost; and the length taken from the rostrum to the end of the caudal rami (not including the caudal setae).

CYCLOPOIDA: SIPHONOSTOMA

Family MICROPONTIIDAE nov.

Only one genus, *Micropontius*, is at present known.

MICROPONTIUS gen.nov.

The characters of the type and only species will serve to define this genus.

Micropontius ovoides sp.nov.

Specimens examined. (1) Numerous adult females, several with ovisacs, and males in magnesium chloride washings (cf. pp. 203–4) from *Spatangus purpureus*, O. F. Müller, trawled or dredged on the Looe–Eddystone and Eddystone Shell Gravel Grounds near Plymouth, March–June 1955. (2) Rather fewer of these from *Echinocardium cordatum* (Pennant), *E. pennatifidum* Norman and *E. flavescens* (O. F. Müller), similarly collected, mainly obtained from the Bigbury Bay and Looe–Eddystone Grounds during the above period. (3) One female from a *Brissopsis lyrifera* (Forbes), dredged 15 miles south of Penmarche Lighthouse (off Concarneau, east coast of France), depth about 53 fathoms; 23 May 1955.

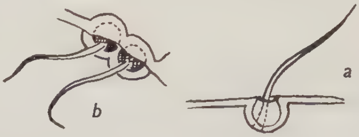

Fig. 1. Detail of the setae on the prosome of *Micropontius ovoides* (semi-diagrammatic). *a*, a view from the side showing the integumental cup, with its narrow opening, and the seta extending to the bottom; *b*, surface view: the ventro-lateral ridge of the segment can be seen to extend as a hood over the outer part of the pit.

Type specimens. The holotype, an adult female, and paratypes, including males and ovigerous females, all selected from the first category above, have been deposited at the British Museum (Natural History). The generic name refers both to size and affinities, the specific to shape.

Description of the female

The ovigerous female is nearly elliptical in outline; a specimen without ovisacs appears somewhat spade-shaped in dorsal view; both nearly twice as long as wide (Fig. 2*a*).

The prosome occupies much the greater part of the body area. The cephalothorax includes the first pedigerous segment, whose anterior limit is indicated only by a thickened strip dorsally and, in part, laterally. There are three metasomal segments, the dorsal articulations of which are also thickened. The postero-lateral corners of all the prosomal segments are produced backward for a short distance into a sharp point; and the posterior border of the third pedigerous is extended to form an 'apron', with a thickened distal edge, which covers the urosome in dorsal view as far as the insertion of the caudal rami. The tergites of the segments bearing the second and third pairs of

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Legend to Fig. 2

Fig. 2. *Micropontius ovoides*, gen. et sp.nov., adult female. *a*, dorsal view (an ovisac and parts of the body, which would otherwise have been obscured, are represented by broken lines in this and other figures); *b*, side view; *c*, antennule; *d*, antenna; *e*, maxilla; *f*, maxilliped; *g*, first swimming leg; *h*, abnormal third endopod segment of first leg; *i*, second swimming leg; *j*, third swimming leg; *k*, fourth swimming leg; *l*, fifth leg; *m*, urosome (detail is shown on one side only).

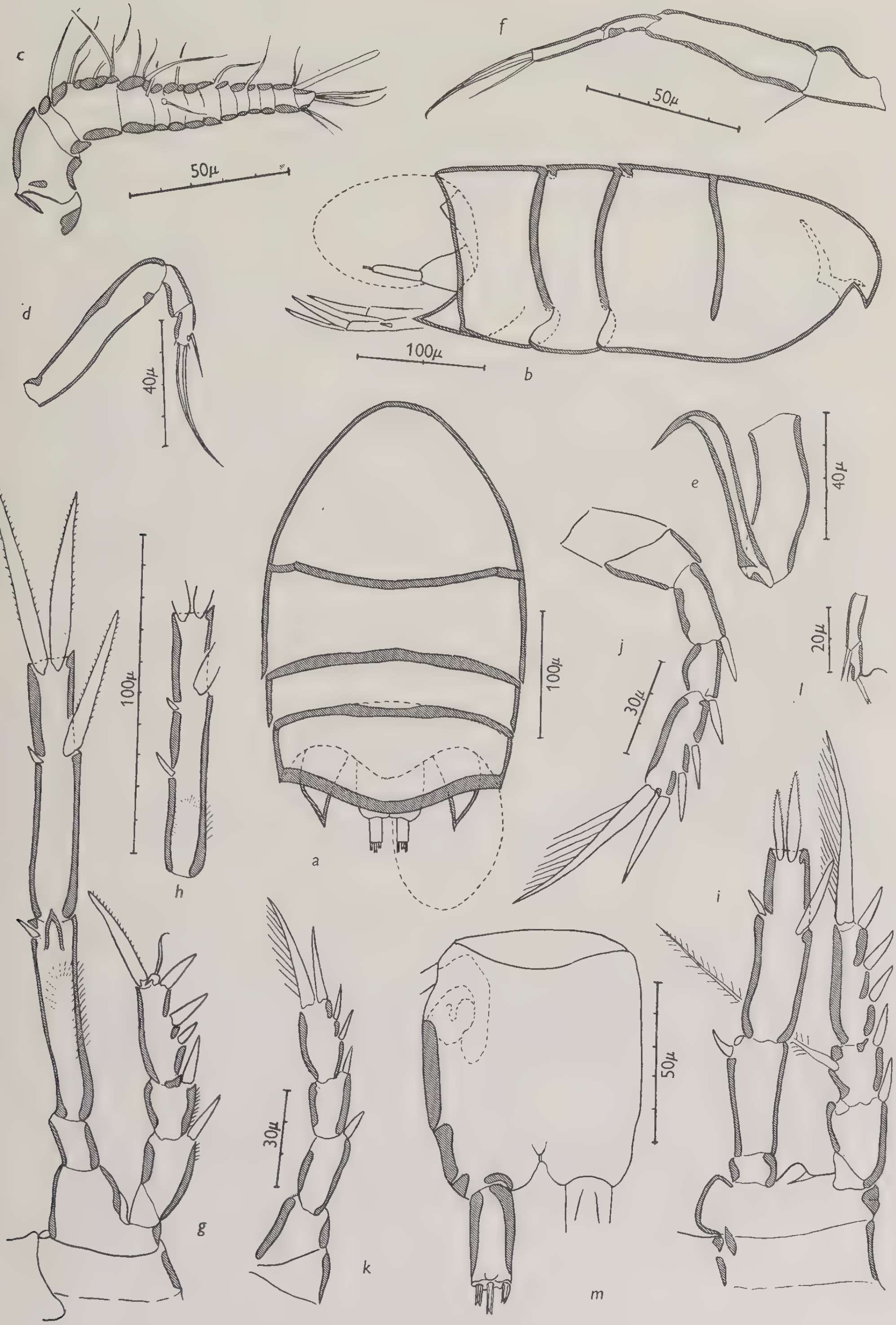
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Fig. 2

legs expand at each end into a slightly concave, ventro-lateral plate (? pleurite) which bears a peculiar seta (cf. p. 203) anteriorly. Five more of these setae occur ventrally on either side of the cephalothorax. The fourth pedigerous somite is more or less fused with the third, but possesses a free, curved lateral end-plate which tapers distally into an acute point.

The urosome (Fig. 2*m*), which includes the somite bearing the fifth pair of legs, is fused into a single subrectangular complex, bearing a pair of caudal rami at the distal end. It is about one-sixth the length of, and slightly more than one-fifth as wide as, the prosome. The lacunae in the rather strong tegumental thickening at the sides suggest that at least two abdominal segments might be found behind the 'genital' somite in an earlier instar.

The rostrum (Fig. 2*b*) has appearance of a stiff beak, curving ventrally.

No eye could be found, even in living specimens.

The antennule (Fig. 2*c*) is 14-segmented; about half the length of the cephalothorax. The shapes, sizes and setation of the segments are shown in the figure.

The antenna (Fig. 2*d*) is 4-segmented and prehensile; the first segment is rather elongate, the last a long, slender, slightly curving claw.

The labrum and labium (cf. Fig. 3*f*) are fused into a (dorso-ventrally) flattened structure, triangular and with flaring postero-lateral corners. The borders are all thickened. The mouth is bulbous and slightly protruding, but there is no siphon. Paragnaths are also absent.

Each mandible consists of a masticatory blade and a palp (cf. Fig. 3*g*). The former is long, tapering and flexible; it protrudes into the mouth through gaps at the sides of the labro-labial complex. The palp is a small, 1-segmented plate which bears terminally a short and a long seta; the two are inserted some distance apart, and not on any well-defined base.

The maxillules are very close to the insertions of the mandibles. Each has a single basal joint, bearing two rami. The latter are of almost equal length; neither, apparently, with a definite articulation between it and the basal segment; the inner is tipped with four setae, the outer with two.

The maxilla (Fig. 2*e*) is 2-segmented; the distal modified into a very strong, hook-like claw which has a hair on either side of, and a single small spine dorsal to, the region of greatest curvature.

The pair of maxillipeds (Fig. 2*f*) are inserted very close together on a basal plate beneath the overhanging distal border of the labium. Each possesses five segments, the last three forming a flexible dactylus, the most distal a slender, very slightly curving claw.

The basipods of all the swimming legs have two well-defined segments; occasionally there is a trace of the pre-coxa. The first and second pairs have each two rami, but in the third and fourth the exopod only is present and resembles that of the first two; all rami are 3-segmented. The endopods of the first pair (Fig. 2*g*) are very long: when extended, they reach beyond the tips of the caudal rami. (In one female specimen an irregularity of the third segment of this ramus was seen: there were two small spines on the outer edge instead of one (Fig. 2*h*).) The fourth pair of legs (Fig. 2*k*) are somewhat smaller than the third (Fig. 2*j*). The ornamentation of the swimming legs is shown in Table 1.

The fifth legs (Fig. 2*l*) are 2-segmented, borne ventro-laterally on the extreme anterior edge of the urosome, extending laterally and curving ventrally. The terminal 'spine' is very similar to those on the caudal rami, and the discussion thereunder is applicable.

There are two ovisacs, each borne dorso-laterally, oval and containing a single egg.

The caudal rami (Fig. 2*m*) are cylindrical, nearly three times as long as wide, and armed terminally—at least in preserved specimens—with three (or occasionally four) short, stout 'spines'. In some specimens which were examined alive and immediately after capture, however, these appeared to be very fine setae about half as long as the urosome; and, if this is the case, they must break during fixing and manipulation.

TABLE 1. ORNAMENTATION OF THE SWIMMING LEGS OF *MICROPONTIUS OVOIDES* SP.NOV.

	Basp.				End.							Exp.						
	1		2		1		2		3			1		2		3		
	Si	Se	Si	Se	Si	Se	Si	Se	Si	St	Se	Si	Se	Si	Se	Si	St	Se
P1	Very small, thread-like setae are irregularly present in some of these positions				0	0	I	0	I	II	I	0	I	0	I	0	I	III
P2					0	0	I	0	I	II	I	0	I	I	I	0	I	III
P3					.	.	.	.	.	.	.	0	I	0	I	0	I	III
P4					.	.	.	.	.	.	.	0	I	0	I	0	I	III

Description of the male

As for the female, except that the body (Fig. 3*a–c*) is an almost perfect ellipse in outline. There is no major articulation, the urosome being firmly fused to the prosome.

The second metasomal segment has no 'apron' projection on its distal edge; the lateral end-plates of the third are fused, near the base to the framework strengthening the insertion of the urosome and more posteriorly to the urosome itself; the two are joined dorsally by a chitinous membrane whose posterior edge is thickened and also fused to the urosome.

The urosome (Fig. 3*a*, *b*) is much wider and more rounded, with a strongly thickened framework. There is no hint of segmentation.

The antennule (Fig. 3*d*) is 13-segmented and doubly geniculate (between the fourth and fifth, and eleventh–twelfth segments). The aesthetasc, on the last segment, is unusually stout.

All the swimming legs (Fig. 3*j–m*) are a little smaller in proportion.

The fifth legs (Fig. 3*n*) are borne ventrally near the middle of the extreme anterior edge, and thus very difficult to distinguish except in a carefully dissected specimen.

A pair of sixth legs are occasionally represented, in well-preserved specimens, by a single small seta at each distal corner of the spermatophoral apparatus.

The caudal rami are inserted rather more ventrally than those of the female (but still visible in dorsal view), and are much shorter.

Dimensions

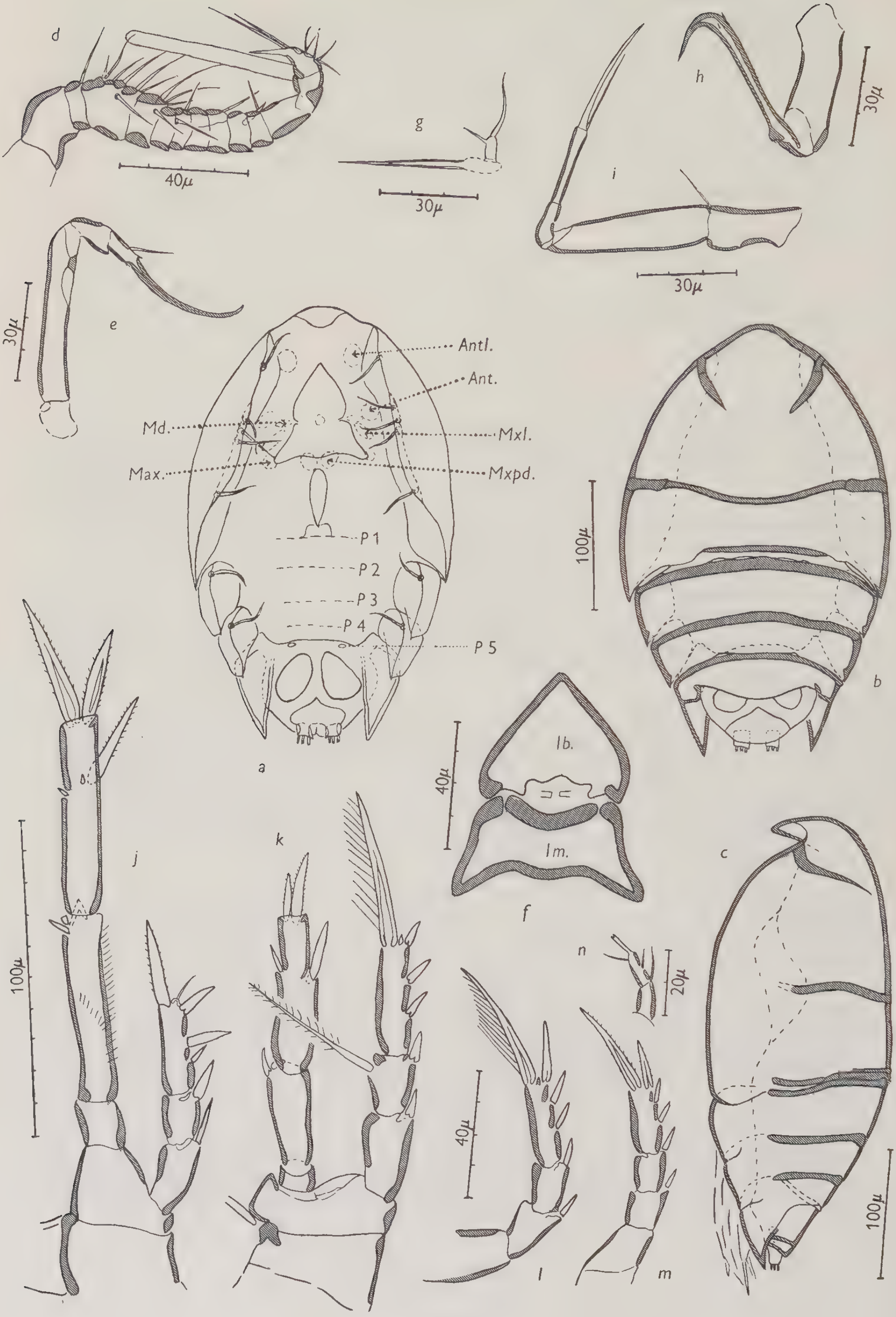
Female—length 0·39 mm; width 0·22 mm. Male—length 0·35 mm; width 0·21 mm.

Colour

This is variable: some specimens exhibiting a reddish tinge, others more or less colourless. The internal organs and ovisacs are usually opaque white to cream coloured; the first arranged so as to leave a clear circular area on either side of the cephalothorax. Occasionally, purplish fragments can be seen in the intestine.

Morphological notes

Three points concerning the external morphology of this species deserve further comment. The first of these is the structure of the extreme posterior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P 3
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k
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l
m
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Fig. 3

part of the body in the female. That this forms a very efficient means of providing support and some degree of protection for the ovisacs may be seen by a comparison of Fig. 2*a* and *b*. The first pair of legs are normally carried fully extended, so that their endopods reach backwards as far as do the ovisacs and support these. The latter are separated dorsally by a prominence just under the distal margin of the 'apron' (cf. Fig. 2*b*), and rather more ventrally by the urosome itself; while the 'apron', both dorsally and laterally, helps to keep them in place.

The second concerns the arrangement of the setae on the ventro-lateral margins of the prosome (Fig. 3*a*). The base of each seta is enclosed in a pit with thick chitinous walls. These pits may often be rather less regular than depicted in both shape and construction—especially in the male, and are situated on the inner side of the ventro-lateral ridge of the segment which continues as a hood over the outer part of the pit, restricting movement of the base of the seta to the shaded portion in Fig. 1*b*. The setae themselves are very long and flexible, and are not plumose. It seems very probable that they have a sensory function though no movement of them could be detected in specimens examined alive. In any case they appear to be unique in structure and position in the Copepoda.

The final note is also related to structures which are probably sensory in function. These have the appearance, in the living specimen, of long, thread-like hairs, but usually form papilla-like projections when preserved. Like the spines on the caudal rami and fifth legs, this may be due to the hardening and subsequent breaking. They are scattered over the exterior of the body, including the rostrum, but are much more frequent on the dorsal and lateral surfaces. Similar structures have been noted while examining specimens of other species, and they are probably present on the majority of, if not all, copepods, being easily overlooked unless the body is minutely examined.

Micropontius ovoides can easily be recognized by a combination of the following characters: body shape and size; arrangement of the pro- and urosomes; lack of the endopods to the third and fourth pairs of swimming legs, and the ornamentation of all the latter; the peculiar setae on the ventral surface; and the mouth shield (labro-labial complex).

Biology

This species (and the other echinoid associates) was obtained by a slight modification of Bocquet's (1952) method; the host was left in a dry dish for

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Legend to Fig. 3

Fig. 3. *Micropontius ovoides*, gen. et sp.nov., adult male. *a*, ventral view, to show where the appendages are inserted (*Antl.*, antennule; *Ant.*, antenna; *Max.*, maxilla; *Md.*, mandible; *Mxl.*, maxillule; *Mxpd.*, maxilliped; *P*1, first, *P*2, second, etc., pairs of legs); *b*, dorsal view; *c*, side view; *d*, antennule; *e*, antenna; *f*, mouth complex (*lb.*, labrum; *lm.*, labium); *g*, the mandible, the basal part is shown by broken line since its extent is not easy to see; *h*, maxilla; *i*, maxilliped; *j*, first swimming leg; *k*, second swimming leg; *l*, third swimming leg; *m*, fourth swimming leg; *n*, fifth swimming leg.

about half an hour, and then sufficient magnesium chloride solution added to cover it completely. (This was the usual $7\frac{1}{2}\%$ solution in fresh water, diluted approximately by half with filtered sea-water; application for a short time seems to anaesthetize most copepods, with the exception of certain harpacticoids). After a period of some 15 min, the liquid was filtered off through fine netting—tied over the nozzle of a large funnel—and the residue washed into a small dish with Plymouth filtered 'outside' sea-water (to avoid introducing extraneous species). The copepods were picked out from this under a binocular dissecting microscope (several were always found trapped in the surface film from which they could be dislodged only with difficulty) and transferred to a dish of clean water, where they recovered rapidly and could be kept alive for some time.

These copepods were found only on the spatangids listed above (p. 198), but no experiments were done on host specificity. That *Spatangus purpureus* is the definitive host at Plymouth is suggested not only by the fact that every one examined over a period of 3 months yielded at least ten, and usually forty or more, *Micropontius ovoides* while specimens of the species of *Echinocardium* never had more than four and were often free from these copepods; but, more particularly, by the following instance: six *Spatangus purpureus*, trawled inside the Eddystone Shell Gravel on 19 May 1955, yielded 289 specimens; an *Echinocardium pennatifidum*, from the same haul and brought in in the same jar, had only two. The water in the jar in this case was also filtered, since it had not proved possible to obtain bottom samples from the *Spatangus* ground for testing to see whether the copepods were confined to the echinoids or generally distributed throughout the substratum, and sixteen specimens were found: most of these proved either dead or moribund.

It is possible, however, that this 'host specificity' may have a correlation with some physical factor, such as particle size of the substrate, *Spatangus*—at Plymouth—preferring a coarse sand and *Echinocardium* spp. a rather more muddy bottom. In this connexion, a *Spatangus raschi* Loven (dredged about 150 miles west of Ushant—48° 05′ N., 08° 11′ W.—in 220 fathoms, 10 April 1955, and kindly identified by Mr George Nicholls, Museum of Zoology and Comparative Anatomy, Oxford) is interesting since, though it was in excellent condition, no copepods were obtained from it; the bottom in this case was a very fine, thick, black mud, with numerous stones. The specimen from *Brissopsis* (bottom marked on chart as 'soft sand, grey mud') is valuable in extending the known distribution of *Micropontius ovoides*, but it cannot be considered an accurate estimate of abundance since the echinoderms were all dead when examined.

To give some idea of the relative abundance of the sexes, a sample from *Spatangus*, obtained on 19 May 1955, was analysed: the 289 specimens included 188 females, of which 46 were ovigerous, and 101 males. No other complete analysis was done, but it is the author's impression that this proportion is about average. The fact that adults are relatively numerous, while only one egg, rather large in proportion to its parents, is ever found in each ovisac, suggests an efficient means of infection and/or a short larval life. Although it was not possible to obtain a satisfactory figure or description of the form which hatches from the egg, it can be stated that this is a perfectly normal siphonostome first naupliar stage, suggesting that if development is abbreviated it must be in the later stages. At no time was anything resembling a late instar found, despite a particularly careful search (since this might have done much to clear up the systematic position of the species).

Careful observations, under magnification (× 24 to 100) made on more than one occasion on hosts which were later proved to be infected, also failed to show any *Micropontius in situ*; but with creatures so small and inconspicuous, in addition to the difficulties of observing a spatangid under such magnification, this cannot be taken as definite proof that the body surface is not their natural habitat. No satisfactory method was devised to test the other hypothesis: that they live in the alimentary or body cavities and move out when the host is dried and immersed in magnesium chloride.

Behaviour in laboratory

Micropontius ovoides usually remains relatively immobile on the bottom of the dish, occasionally working its mouthparts or making quick jerks with its swimming legs. It always comes to rest on its back, and seems unable to swim off from this position.

When a spatangid spine is introduced, however, and the creature manoeuvred until it has grasped this, it becomes rather more active. The antennae and maxillipeds are used both to grip the spine and to keep the body clear of it; the swimming legs are held close to the body surface; the antennules usually at right angles to the body and above the substrate. Occasionally, when at rest, the aesthetasc is used, apparently to palpate the spine. When 'walking', the antennae and maxillipeds move in a typically quadripedal succession, and the creature may scurry up and down the spine quite rapidly.

Attempts to dislodge a specimen from a spine, for example by gentle nudges with a needle, may produce any of four responses: scuttling away to another part of the spine; 'freezing', in which the body is pressed close to the spine, the maxillae probably (this could not be observed) being used to provide greater gripping power; transference to the needle point; or an 'annoyance' reaction, when the animal swims up and away from the spine with rapid jerks of its swimming legs and then sinks motionless until it lies on the bottom, ventral surface uppermost. Since the creature seems unable to swim off except from some support, it is possible that the long endopods of the first legs, with or without assistance from the antennae or maxillipeds, are used to give a 'shove off'.

Specimens pipetted on to a living *Spatangus* and observed under a binocular dissecting microscope did not behave in any way differently from that described above. Most remained holding on where they fell—occasionally, if they were on a spine, moving up or down it—and exhibiting the usual reactions when touched with a needle. None ever left the host, or swam to a different spine, or, when near the mouth, attempted to enter this.

Systematics

The systematic position of the new genus *Micropontius* is doubtful. It is certainly siphonostome, and, as the Siphonostoma stand at present, it possesses many characters which suggest relationship with the Dyspontiidae and Asterocheridae. As Nicholls (1944, pp. 15–16) points out, the former is not a very well-defined family; and there are several anomalous genera, such as *Stephopontius* Thompson & Scott (referred by Sewell, 1949, p. 167, to ? Cancerillidae), which he does not include in it but which exhibit certain resemblances both to the Dyspontiidae and to this new genus. My opinion is that *Micropontius* is sufficiently distinct to be separated, for the present, as the type of a new family, the Micropontiidae, but, since a future (and much needed) revision of the Siphonostoma may uncover possible evolutionary lines into which it will fit, I have decided not to expand this idea further in the present paper.

Family ASTEROCHERIDAE Giesbrecht, 1899 (*sens. strict.*)

Asterocheres violaceus (Claus, 1889)

Occurrence. Numerous adult males and females were found in magnesium chloride washings of *Echinus esculentus* L., obtained from various localities in the region, throughout the year. Specimens averaged about fifty per host; with a sex ratio of nearly two females to every male. Instars from 'Copepodid II' (i.e. an immature specimen with three pairs of legs and the rudiments of a fourth) to adult occurred rather rarely in March and April 1955.

Distribution. North Sea to Mediterranean; adults (? and stages) typically associated with camarodont echinoids, but occasionally with asteroids or free from a host.

Notes. I have followed Giesbrecht (1899) in regarding the genus *Echinocheres* Claus as a synonym of *Asterocheres* Boeck, since the distinction between them seems too slight to justify retention of the former. In support of this view, it may be mentioned that in my specimens, as in those of A. Scott (in Herdman & Scott, 1896)—which both Giesbrecht (1899) and Sars (1913–18) agree in referring to this species—the mandibular palp was distinctly 2-jointed and the fifth pair of legs in the female ciliated on both sides; this appears to leave only the lengths of the siphon and maxillular setae—surely characters of specific rather than generic importance?

Family DYSPONTIIDAE Sars, 1915

Scottomyzon gibberum (T. & A. Scott, 1894)

Occurrence. One male was found in a jar containing *Spatangus purpureus* Müll., dredged inside the Eddystone Shell Gravel Grounds, 19 April 1955.

Distribution. Norway to English Channel; adults typically found associated with *Asterias rubens* L.

Notes. Several *A. rubens* were searched at various times throughout the year, but the 'loges irrégulières' mentioned by Bocquet (1952) were not found, nor did washings from the surface yield any of these copepods.

CYCLOPOIDA: POECILOSTOMA

Family LICHOMOLGIDAE Kossmann, 1877

Lichomolgus leptodermatus sp.nov.

Specimens examined. Several males and females (most of the latter ovigerous) from the gills of a single *Laevicardium crassum* (Gmelin) trawled on the Looe–Eddystone—'Two-in-one' Grounds, 8 March 1955.

Type specimens. The holotype (an adult female) and several paratypes (both males and females), all cleared in lactic acid have been deposited at the British Museum (Natural History).

Description of the female

The body shape (Fig. 4*a*) is cyclopoid, nearly three times as long as wide; urosome about equal in length to the prosome, one-third its width.

The prosome is oval in dorsal outline, nearly one and a half times as long as broad; slightly inflated. The dorsal plates of the prosomal segments are separated laterally, and rounded at the corners. The cephalothorax includes the first pedigerous segment, the line of fusion being indicated by a groove dorsally and laterally.

The urosome is 5-segmented, though there is a flexible intersegmental region between the genital and fifth pedigerous segments. The genital is the widest segment in the urosome, and is divided dorsally (but not ventrally) by a distinct line; it is spindle-shaped. The succeeding abdominal segments are all alike in size.

A spoon-shaped rostrum (Fig. 4*b*) is present, which extends forward and down, and is not prehensile.

There is also an eye.

In the 7-segmented antennule (Fig. 4*c*), the articulation between the third and fourth segments is indistinct. There are not very many setae, and the representation of these in the figure is approximate only, since, even when stained, they are very difficult to make out. No aesthetasc could be seen.

The antenna (Fig. 4*d*) is 4-segmented, the third being small and indistinctly defined. The appendage is weakly prehensile, the terminal segment tipped with a curved and jointed claw and two weaker but unjointed ones, the inner the longest.

The labium is inflated and cone-shaped (in lateral, anterior and ventral views). The posterior edge has a deep, even groove down the mid-line.

No paragnaths could be found.

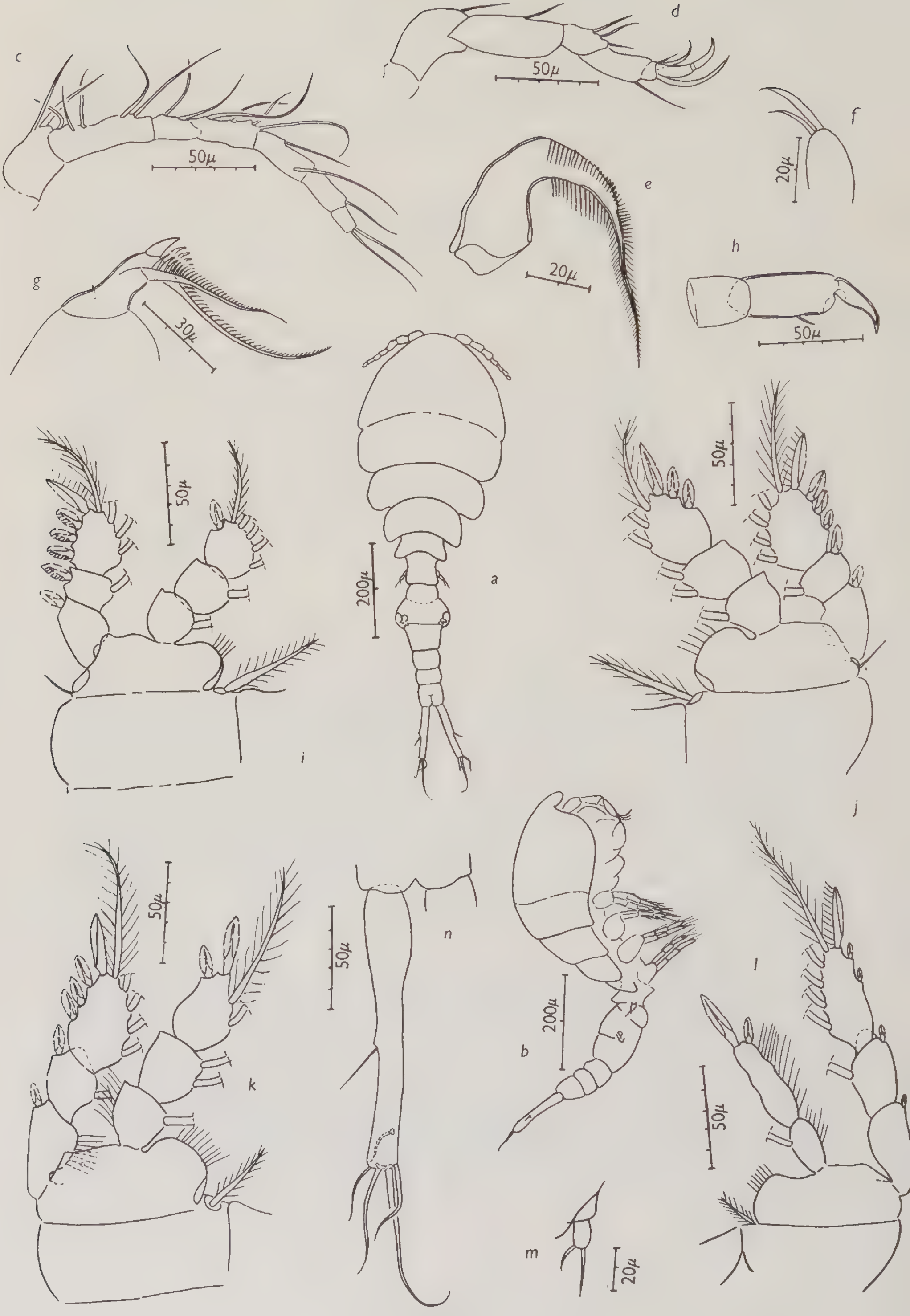
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Fig. 4

The labium is so indistinctly defined that no details of size, shape or texture could be made out.

The mandible (Fig. 4*e*) is of the usual lichomolgid type, with a single long, tapering lappet which is finely spinose on either edge; none of these spinules is enlarged.

There is a small, palp-like maxillule (Fig. 4*f*) with two rather stout terminal setae of differing lengths.

The basal segments of the 2-segmented maxilla (Fig. 4*g*) is rather short and inflated. The distal has a terminal, seta-like blade which is spinose—the spinules becoming longer and stronger towards the base, the proximal one very stout—on the inner edge only; and a shorter, but otherwise very similar, accessory blade borne a short distance towards the 'outside' (considering the part *in situ*). There is a small spine near the base of the segment.

The maxilliped (Fig. 4*h*) is also 2-segmented, with a strong complex attachment to the framework of the head. The first segment is rather long, with a small seta on the inner side; the last modified into a stout, but not very strong, claw.

The swimming legs (Fig. 4*i–l*) have each a basipod of two segments, and two rami, all 3-segmented except the endopod of the fourth legs which has two only. Their ornamentation is illustrated in the figures mentioned above, and summarized in Table 2.

TABLE 2. ORNAMENTATION OF THE SWIMMING LEGS OF *LICHOMOLGUS LEPTODERMATUS* SP.NOV.

	Basp.				End.							Exp.						
	1		2		1		2		3			1		2		3		
	Si	Se	Si	Se	Si	Se	Si	Se	Si	St	Se	Si	Se	Si	Se	Si	St	Se
P1	I	0	0	1*	1	0	1	0	5	0	I	0	I	1	I	4	I	III
P2	I	0	0	1*	1	0	2	0	3	I	II	0	I	1	I	5	I	III
P3	I	0	0	0	1	0	2	0	2	I	I	0	I	1	I	5	I	III
P4	I	0	0	0	1	0	0	0	.	.	.	0	I	1	I	5	I	II
							II	(St)										

* This may occasionally be absent.

The fifth pair of legs (Fig. 4*m*) are very small; 2-segmented, the basal fused with but sharply defined from, the thoracic somite, and borne midway along this. There is no seta on the basal segment, but two terminally on the distal, the inner shorter and thicker.

A small seta and a curving spine laterally near the middle of the genital segment represent all that could be seen of the sixth legs.

The caudal rami (Fig. 4*n*) are very elongate, about as long as the post-genital segments together, and eight or nine times as long as wide. There is an outer-edge seta just in the distal half, and four terminal ones—the longest only five-eighths the length of the ramus, none setose—and a small dorsal seta near the distal border.

Legend to Fig. 4

Fig. 4. *Lichomolgus leptodermatus*, sp.nov., adult female. *a*, dorsal view; *b*, side view; *c*, antennule; *d*, antenna; *e*, mandible; *f*, maxillule; *g*, maxilla; *h*, maxilliped; *i*, first swimming leg; *j*, second swimming leg; *k*, third swimming leg; *l*, fourth swimming leg; *m*, fifth leg; *n*, dorsal view of the left caudal ramus.

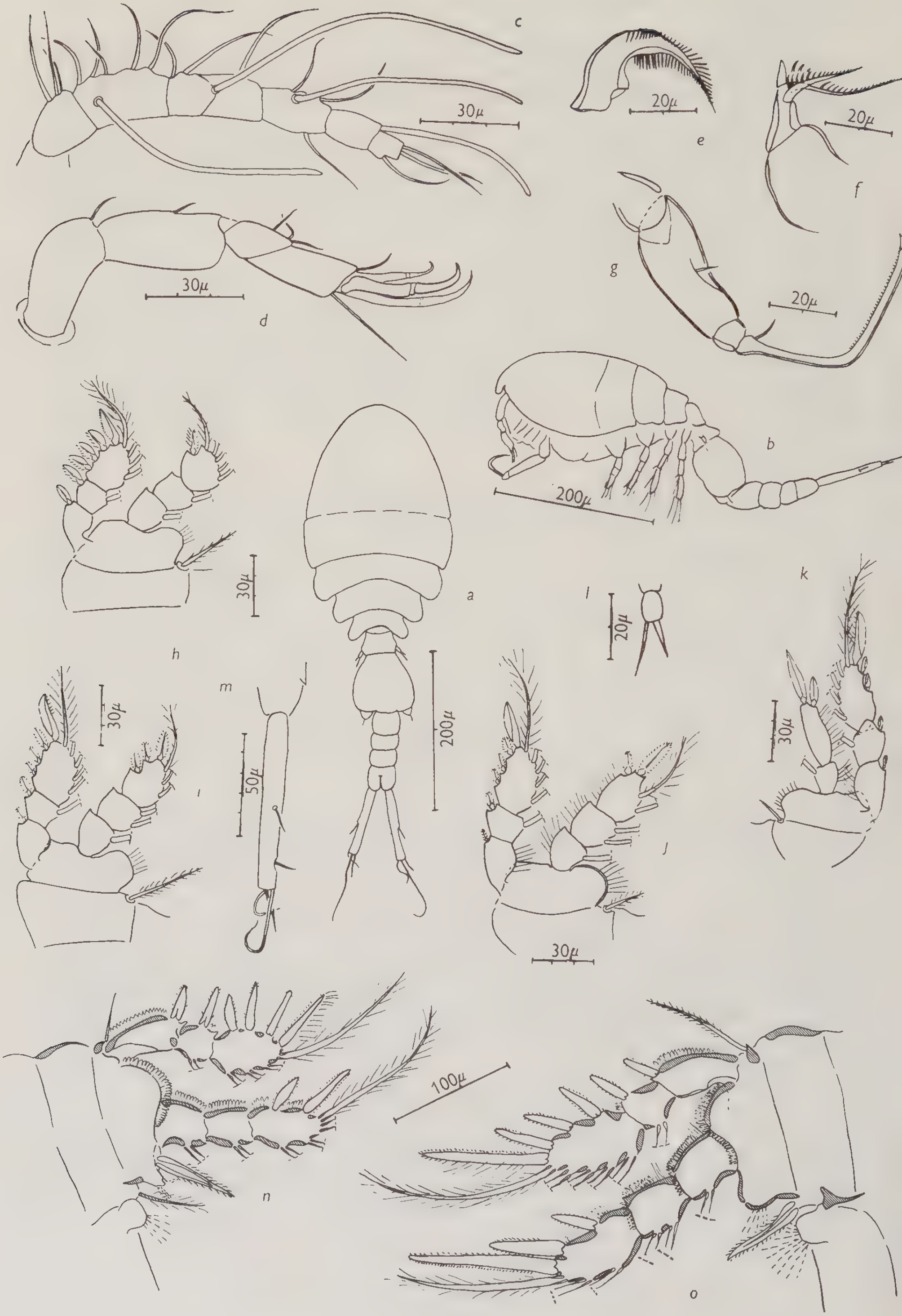
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Fig. 5

Description of the male

As for the female, except the body shape (Fig. 5 *a*) is slightly more elongate—more than three times as long as wide. This is due, in great measure, to a lengthening of the prosome, so that the urosome attains more than one-third the width of this. The junction of the first pedigerous segment with the rest of the cephalothorax is not very well-defined.

The urosome is 6-segmented, with no intersegmental region; it is four times as long as wide. The genital segment broadens from front to rear, and is undivided.

The antennule (Fig. 5 *c*) has an aesthetasc on each of the second, fourth, fifth and sixth segments.

All three terminal claws of the antenna (Fig. 5 *d*) are jointed, but this is less clear in the outer and inner ones.

The mouthparts (Fig. 5 *e–f*) have a slightly stronger integument.

Of the three free segments of the maxilliped (Fig. 5 *g*), the last is a very long, narrow claw, bent in the centre almost into a right-angle, the distal half with a row of very fine hairs on the inner side; there is a short seta near the base of this segment.

The setae on the outer side of the second basipod segment, present in the first and second pairs of swimming legs in the female, may be lacking.

The sixth pair of legs are represented by a single small seta on each postero-lateral corner of the genital segment; these appear in dorsal view to be borne on a small mammiform projection.

Dimensions

Female, length 0·90 mm, greatest width 0·33 mm. Male, length 0·59 mm, width 0·19 mm.

Colour

Colourless and transparent except for the ovary, ovisacs and testes which were opaque by transmitted, and white (in the case of the first two) or pale rose by reflected light; and the ruby-red, shining eye.

Morphological notes

A most noticeable feature of the living animal was the posture of the body. Even when swimming freely, this was bent sharply between the fourth and fifth pedigerous segments (the major articulation) and again between the genital and succeeding abdominal segments—roughly like a 'Z' which had been pulled out at either end until the angles between the parts were obtuse. (An attempt to show this has been made in Fig. 5 *b* but the original drawing was made on a lactic acid-cleared specimen, in which the exoskeleton was too flexible to hold its original shape, and this necessitated some alterations to obtain the desired effect.)

Legend to Fig. 5

Fig. 5. *a–m. Lichomolgus leptodermatus*, sp.nov., adult male. *a*, dorsal view; *b*, side view; *c*, antennule; *d*, antenna; *e*, mandible; *f*, maxilla; *g*, maxilliped; *h*, first swimming leg; *i*, second swimming leg; *j*, third swimming leg; *k*, fourth swimming leg; *l*, fifth leg; *m*, side view of caudal ramus. *n–o. Conchyliurus cardii*, sp.nov., adult female. *n*, first swimming leg; *o*, second swimming leg.

Systematics

The nearest attempt at a key to the genus *Lichomolgus* Thorell is to be found in Sewell's (1949, pp. 93-94) analysis of the setal formulae in the various species. *L. leptodermatus* represents a variation of that author's 'final stage of reduction', since the suppression of spines has extended to the third pair of swimming legs where one has been lost on the terminal segment, not of the exopod, but of the endopod.

In addition, the following characters appear to be distinctive, either separately or in conjunction: the very large proximal spine on the main lappet of the maxilla; the proportions of the caudal rami and their setae; the terminal armature of the antenna; the dactylus of the male maxilliped; and the very thin exoskeleton. It will be seen that the species does show a resemblance to *L. albens* Thorell.

Biology

The specimens were all found in small swellings of the gill tissues of the host. Each of these 'cysts' contained usually one male and one female, but as many as two females and three males did occur together. Both the large and small gills were infected, and these on either side (the swellings showing up clearly as whitish spots against the pink of the gill tissue); but neither the mantle nor the labial palps yielded any; nor were any seen swimming in water taken from the mantle cavity.

No movement could be detected in specimens lying in these swellings but, when they were released, there was a brief initial period of intense activity, usually terminating when the creature came to rest on surface of the gill. The males were, as usual, more active than the females. In a dish, this alternation of periods of activity with long quiescent phases—provided no sudden external stimulus was applied—persisted; under these conditions, too, ovigerous females soon shed their ovisacs.

Some of the specimens collected must have been in the last copepodid stage since cast skins were found in a culture which had been kept overnight; but these moult stages proved too delicate for manipulation and no immature specimens were found on subsequent re-examination. Infection of *Laevicardium* may take place in one of the later copepodid stages, as a careful search of the gills failed to discover any early instars.

Pseudanthessius liber (Brady & Robertson, 1875)

Occurrence. This species was found several times, but only in small numbers (one or two per host), together with *Asterocheres violaceus* in washings from the surface of *Echinus esculentus*, January and February 1955.

Distribution. Coasts of Norway, British Isles and Channel, Indian Ocean; adults typically associated with camarodont echinoids, but occasionally taken from other invertebrates or not associated.

Family CLAUSIDIIDAE Embleton, 1901

Conchyliurus cardii sp.nov.

Specimens examined. Eight females—two samples of three and five respectively—all ovigerous, were found in jars containing *Cardium* (*Acanthocardia*) *echinatum* Linn. dredged in Bigbury Bay, near Plymouth; 23 May 1955 and 6 July 1955.

Type specimens. Holo- and paratypes have been deposited in the British Museum (Natural History). The specific name refers to the host.

Description of the female

The body (Fig. 6*a*) is rather more elongate than the typical cyclopoid form; and strongly built. The prosome is little expanded, its dorsal outline forming an ellipse (truncated posteriorly), whose long axis would be (if not truncated) about twice the short. The greatest width is in the region of the first pedigerous segment; this somite is included in the cephalothorax; and there is no trace of a line of fusion between it and the rest of the 'head' region. The cephalothorax occupies about half the prosomal area. There is a band of very fine spinules just outside its ventro-lateral border. The lengths of the three metasomal segments are about equal; the postero-lateral corners of the first two and the cephalothorax appear slightly produced and pointed in dorsal (or ventral) view.

The urosome is 5-segmented, with a flexible intersegmental region between the fifth pedigerous and the genital segments. (This has the appearance of another somite, and is marked off with equal distinctness from both the foregoing. It is probably, however, part of the fifth pedigerous segment: the integument is not thickened as it is in the other urosomal segments, and in dead specimens the body is usually bent sharply at this point, cf. Fig. 6*b*). The segment bearing the fifth pair of legs is rather narrower than that preceding it, and not very long. The genital segment is the widest in the urosome, but slightly longer than wide. It is structurally very complex, and more material will be necessary before all the details can be satisfactorily elucidated. The anal segment is equal in length to the two preceding abdominal somites, and has a row of very fine teeth ventrally near the proximal end and similarly on the distal border. The widths of these three abdominal segments decrease evenly from front to rear.

The rostrum (Fig. 6*c*) is rather well-developed; it is visible in dorsal or ventral view rather than lateral (where it tends to be concealed by the antennules), and apparently non-mobile.

There is a double kidney-shaped eye, which can be seen in the living animal to have a shining spot (? lens) on the anterior end of either side.

The antennule (Fig. 6*d*) extends only about half the length of the cephalothorax. It is 6-segmented; the relative lengths, widths and armature of the segments are shown in the figure, and there are aesthetascs on the fifth and seventh segments.

The antenna (Fig. 6*e*) is a strongly prehensile, 4-segmented structure, with a large claw on the penultimate segment and seven articulated processes terminally. The surfaces of the segments are covered with patches of denticles, some small and spine-like, others—especially on the second and third segments—larger and more robust. In addition to the claw on the distal corner of the third segment, there is a small toothed projection near whose base a short seta is borne. The terminal segment is, as usual in the Clausidiids, somewhat offset.

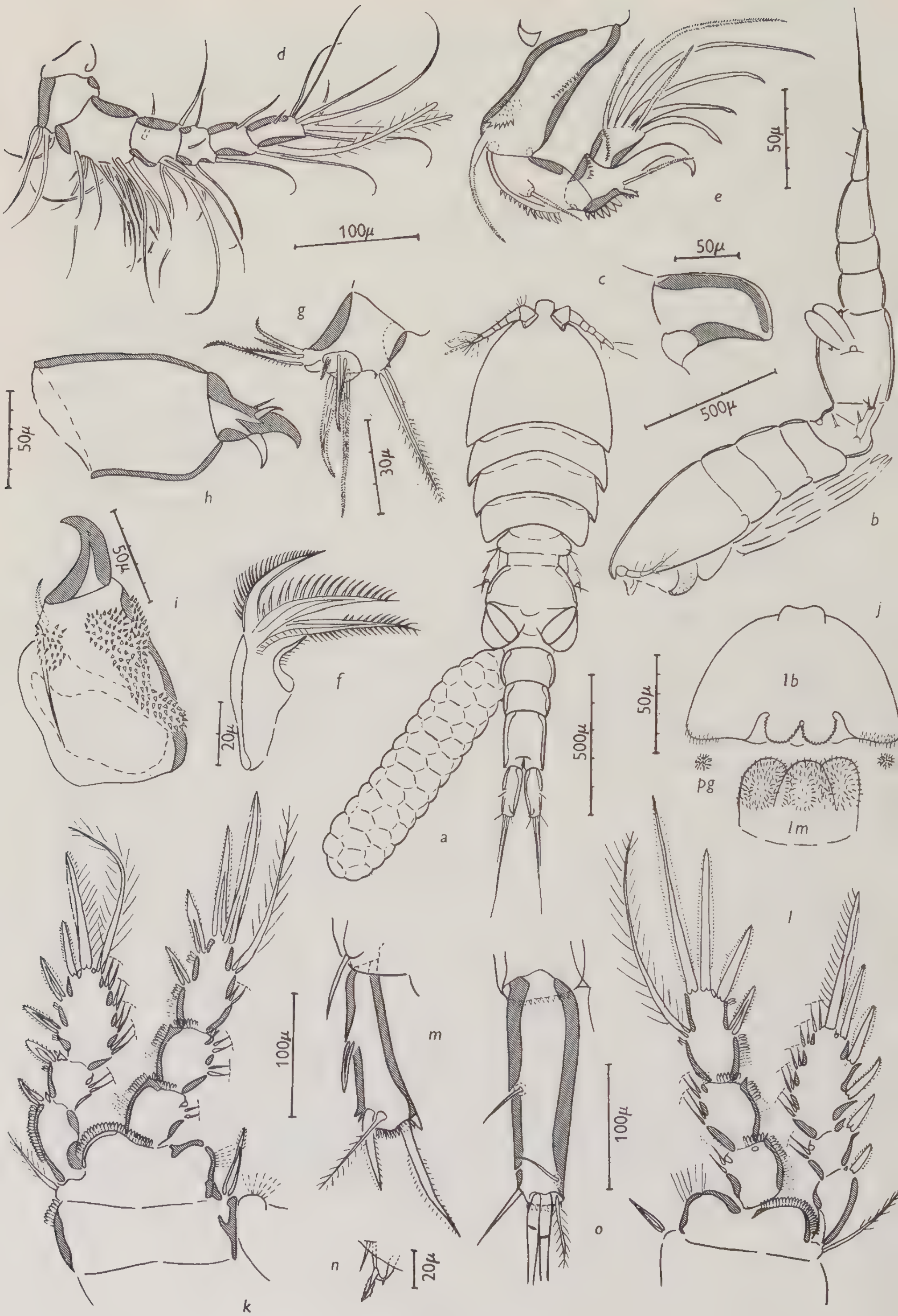
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Fig. 6

The mouthparts are all very closely appressed to each other. The labrum (Fig. 6*j*) has a sinuate posterior margin, beneath which are two small serrate lobes, one on either side of the mid-line. The paragnaths are rudimentary, being represented by small ciliated projections between the mandibles and maxillae. A small, indistinct, spinose structure, with two grooves dividing it into three aproximately equal lobes represented all that could be identified as the labium.

The mandible (Fig. 6*f*) has three lappets, almost equally elongate although the fact that the two upper ones are usually curved makes the lowest seem longer; these are all borne on a thickened base, the innermost partly joined to a prominence. The middle lappet is coarsely setose, the others more finely.

The maxillule (Fig. 6*g*) consists of a single free segment, partly bi-lobed. Its shape and ornamentation are as illustrated.

The maxilla (Fig. 6*h*) is 2-segmented; the first large and easily seen in ventral view: the second is modified into a very strong, stout, curved claw which bears two smaller claws, one on the concave surface and the other to one side, usually projecting above the large claw. The first segment has no ornamentation.

TABLE 3. ORNAMENTATION OF THE SWIMMING LEGS OF *CONCHYLIURUS CARDII* SP.NOV.

	Basp.				End.							Exp.						
	1		2		1		2		3			1		2		3		
	Si	Se	Si	Se	Si	Se	Si	Se	Si	St	Se	Si	Se	Si	Se	Si	St	Se
P1	1	0	I	1	1	0	1	0	2	2	II	0	I	1	I	4	I	III
P2	I	0	0	1	1	0	2	0	2	I	II	0	I	1	I	5	I	III
P3	I	0	0	1	1	0	2	0	2	II	II	0	I	1	I	5	I	III
P4	I	0	0	1	1	0	2	0	1	II	II	0	I	1	I	5	I	III

The maxilliped (Fig. 6*i*) is very similar to the maxilla. The first free segment, however, has patches of small spinules on the posterior (considering the appendage *in situ*) surface, and a short seta near the distal edge. The other segment is, like that of the maxilla, modified into a strong uncinate claw which lacks secondary claws but has, on either side in the region of greatest curvature, a small, stout denticle.

The last three pairs of mouthparts project ventrally more or less at right angles to this surface of the cephalosome, the maxilla and maxilliped curving distally in towards one another.

A 'postoral protuberance' (Humes, 1953, p. 101), similar to that present in many copepods, can clearly be seen in lateral view (Fig. 6*b*). It is an inflated region, with thin integument and an almost square base, located between the maxillipeds and first swimming legs.

The swimming legs (Figs. 5*n*, *o*, 6*k*, *l*) have all 2-segmented basipods and two rami, each of three segments. The ornamentation is shown in Table 3 and figures. It is interesting to note that the seta on the proximal basipod segment of the first legs is replaced in this position on the other swimming legs by a spine. The outer and distal edges of several of the segments have a row of very small cross-set teeth and/or hairs. The basal

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Legend to Fig. 6

Fig. 6. *Conchyliurus cardii*, sp.nov., adult female. *a*, dorsal view; *b*, side view; *c*, rostrum; *d*, antennule; *e*, antenna; *f*, mandible; *g*, maxillule; *h*, maxilla; *i*, maxilliped; *j*, mouth complex (abbreviations as for Fig. 3*f*, plus *pg*, position of paragnath); *k*, third swimming leg; *l*, fourth swimming leg; *m*, fifth leg; *n*, sixth leg; *o*, dorsal view of caudal ramus. In *a* and *b* spermatophores can be seen.

plates (lamellae joining the basipods of each pair of legs) possess a rounded lobe, with a tuft of hairs, distally at either end; and the second basipod segment of the first swimming legs has a pointed prominence on the inner side.

The fifth pair of legs (Fig. 6*m*) is 2-segmented, the basal being completely fused with the thoracic segment and represented only by a widening of this and a seta. The latter is borne on a small papilla, rather more dorsal to the insertion of the free segment than is usually the case (cf. Fig. 6*b*). The distal segment is more or less triangular with almost straight sides; about four times as long as wide; with three spines—two more or less terminal, a plumose seta between them, the inner the longer—and one on the outer edge. The terminal margin has a row of close-set spinules, the inner- and outermost rather larger and longer.

The sixth pair of legs (Fig. 6*n*) are represented by a pair of projections on the dorsal surface of the genital segment: they are concealed for most of their length by the attached spermatophores. Each has a long feathered spine terminally and another (unornamented) near the base.

The ovisacs (Fig. 6*a*) are rather longer than the urosome, and relatively stout; borne laterally from the middle of the genital segment. Each contains about a hundred eggs.

The caudal rami (Fig. 6*o*) are nearly five-sixths the length of the anal segment and, like the latter, each has a row of very fine teeth ventrally on the distal border. The setae are as illustrated: the two longest with no other ornamentation than a very finely dentate border and a narrow wing for the distal three-quarters of their length.

Dimensions

Length 1·83 mm; greatest width 0·48 mm.

Colour

Colourless, with opaque whitish spots; the ovaries, oviducts and egg-sacs showing rose-pink, and the alimentary canal and region of the fused spermatophores brown.

Male. Unknown.

Biology. The external morphology suggests that this species is an associate, but specimens were never found actually in contact with a *Cardium*, always in the water surrounding it. A careful search of gills and mantle cavity in both samples of specimens (and one from the Looe–Eddystone Grounds in which no copepods occurred) being negative, it is suggested that future examination of the digestive system might be valuable. In any event, it is unlikely that the

TABLE 4. DIFFERENCES BETWEEN ADULT FEMALES OF *CONCHYLIURUS SOLENIS* AND *C. CARDII*

	C. solenis Bocquet & Stock	*C. cardii*, sp.nov.
1.	Accessory hooks on maxilla poorly developed	Accessory hooks on maxilla well developed
2.	Knob on inner side of basipod P 1 rounded	Knob on inner side of basipod P 1 pointed
3.	No process on outer distal edge of P 5	Large spinule on outer distal edge P 5
4.	No spines on anal segment	A line of fine spines on ventral surface of anal segment both near the proximal and on the distal border
5.	Spermatophores not retained on genital segment	Spermatophores retained and fused to genital segment in a complex fashion
6.	Specimens found on gills of host	Specimens probably in digestive tract of host

specimens were associated with any other host, since the jars in which they were found contained only this species of *Cardium* and were covered, immediately after the molluscs were introduced, with fine netting.

Remarks. Bocquet & Stock (1957) erected the genus *Conchyliurus* for a species (*solenis*) which was found on the gills of *Solen marginatus* near Roscoff. Thanks to the kindness of the latter author, I was able to compare paratypes of this with my specimens. The two are very similar; indeed, it is open to question whether they might be considered host or geographic variations of a single species. However, I have decided to separate mine as a new species by the differences shown in the table, since these seemed both consistent over the range of specimens and of more than phenotypic character. It is hoped that a future comparison of material of both species from a particular locality will determine whether this is the best statement of their relationship.

Family Bomolochidae Wilson, 1911

Bomolochus confusus Stock (1953)

B. soleae auct., non Claus

Occurrence. Adult females and males occurred in the nostrils of the Cod (*Gadus callarias* Linn.), Pout (*G. luscus* Linn.), and Whiting (*G. merlangus* Linn.), all trawled in the Plymouth region. Incidence figures are not given: they vary markedly, depending on whether the hosts are examined immediately on capture or after being brought into the laboratory. However, at sea, nearly every *G. luscus* of moderate size had at least one female—with a maximum of five—in each nostril; in the laboratory, an average figure for *G. merlangus* was one female to every eight hosts; while only one of several *G. callarias* examined had this associate. Males were very rare.

Distribution. British coasts; adults in nostrils of gadoid fishes.

Notes. This species was distinguished by Stock (1953) from *Bomolochus soleae* Claus mainly on differences in structure of the antennae, maxillipeds and swimming legs—especially the second pair. The specimens I have examined (from the three hosts mentioned) support his distinction, but seem to show minor differences from one another; this suggests that it might be possible to distinguish host-specific forms on morphological grounds.

CYCLOPOIDA: GNATHOSTOMA

Family Cyclopidae Sars, 1913

Euryte longicauda Philippi (1843) var. *minor* T. Scott (1905)

Occurrence. One ovigerous female was found in washings from clinkers and pieces of bored limestone dredged on Asia Shoal, Plymouth Sound, 14 March 1955.

Distribution. Var. *minor* occurs in the North Sea and English Channel, adults not associated with animals. Typical *E. longicauda* has been recorded from the Arctic Ocean, Atlantic coasts of Europe, British Isles, Mediterranean, Black Sea and Suez Canal; adults littoral.

Notes. Kiefer (1929) includes this form in *E. longicauda*, but Lang (1946*b*, pp. 1, 2) distinguishes it as a separate species by the relatively longer abdomen and divergent caudal rami. I think Scott's intermediate course is the best until some experimental work has been done on the relationship between these two forms.

CALIGOIDA

Family LERNAEOCERIDAE Gurney (1933)

Peniculus fistula Nordmann (1832) f. *pagelli* Delamare Deboutteville & Nunes (1951)

Occurrence. (1) One female on the caudal fin of the Common Sea Bream (*Pagellus centrodontus* de la Roche), hand-lined near the Eddystone Lighthouse *ca.* 15 fm; June, 1954. (2) One female on the pelvic fin, one ovigerous female on the caudal fin of a Spanish Sea Bream (*P. erythrinus* Linn.) removed from the Plymouth Aquarium; October 1954. (This fish had been trawled on the Mewstone Grounds and kept in the Aquarium for some time. It is probable that it was infected before capture.) (3) One female on the caudal fin of *P. centrodontus*, trawled on the Looe–Eddystone Grounds; July 1955.

Distribution. The species is known from the English Channel to the Mediterranean, the adult female on a variety of hosts (Candeias, 1955). Forma *pagelli* occurs through this range, the adult female on the fins of *Pagellus* sp.

Notes. There seems some difference of opinion about the authorship of the name *Peniculus fistula*. Nordmann (1832), who described the species, mentions (p. 107) that his specimens had been found by Rudolphi in 1817. The latter (he says) had sent these, together with unpublished notes and a suggested name (*Dirhynchus fistula*), to him; he changed the generic name, since it was already in use, but retained the specific for the purpose of his description. According to the Rules of Nomenclature, therefore, Nordmann should be credited with this name.

My specimens agreed closely with the description and figures of f. *pagelli* given by its authors except that the lengths, even of the ovigerous female, were slightly less. Also, each exhibited a slight curvature of the 'neck' so that the main part of the body was in life held parallel to, and slightly above the surface of the fin to which the head was attached.

This appears to be the first record of the genus in Britain; and of *Pagellus centrodontus* as host for this form.

HARPACTICOIDA: OLIGOARTHRA

Family TISBIIDAE Stebbing (1910) *sensu* Lang (1948)

Tisbe elongata (A. Scott, 1896)

Occurrence. All stages from the 'Nauplius V' (that is, a stage corresponding to the one described as Nauplius V for *T. furcata* by Johnson & Olson, 1948) to adults were found among the gills of the common lobster (*Homarus vulgaris* M. Edwards) at Plymouth. The copepods were very abundant, averaging nearly fifty per host; and each lobster examined over a period of about a year was infected. In August 1953, also, while working for the Scottish Home Department's Fisheries Laboratory in Aberdeen, I found this species to be very common on lobsters in the Orkneys.

Distribution. British Coasts; adults and stages typically found on gills of *H. vulgaris.*

Notes. This record is included, although the species has already been noted from Plymouth, both to extend the distribution (by the Orkneys record) and, by emphasizing their abundance and the extent to which developmental stages occur on lobster gills, to support Gurney's (1933, p. 299) suggestion that the species is definitely associated with *H. vulgaris* rather than 'usually free-living' (Humes, 1954).

SUMMARY

The adults of three new cyclopoid copepods are described and figured, namely *Micropontius ovoides*, gen. et sp.nov., occurring on spatangid echinoids; *Lichomolgus leptodermatus*, sp.nov., and *Conchyliurus cardii*, sp.nov., both associated with pelecypod molluscs. *Micropontius* is made the type of a new family, the Micropontiidae.

Some other copepods, *Asterocheres violaceus* (Claus), *Scottomyzon gibberum* (T. & A. Scott), *Pseudanthessius liber* (Brady & Robertson), *Bomolochus confusus* Stock, *Euryte longicauda* Philippi var. *minor* T. Scott and *Peniculus fistula* Nordmann, are recorded for the first time from the Plymouth region; and mention is also made of *Tisbe elongata* (A. Scott).

Points of interest in the biology, morphology, systematics and distribution of all these are discussed.

REFERENCES

ALLEE, W. C., EMERSON, A. E., PARK, O., PARK, T. & SCHMIDT, K., 1949. *Principles of Animal Ecology.* 837 pp. Philadelphia: Saunders.

ATKINS, D., 1934. Two parasites of the Common Cockle *Cardium edule*; a rhabdocoele, *Paravortex cardii* Hallez and a copepod *Paranthessius rostratus* (Canu). *J. mar. biol. Ass. U.K.*, Vol. 19, pp. 669–76.

BAER, J. G., 1951. *Ecology of Animal Parasites.* 224 pp. University of Illinois Press.

BOCQUET, C., 1952. Copépodes semi-parasites et parasites des Echinoderms de la Région de Roscoff. Description de *Lichomolgus asterinae* n.sp. *Bull. Soc. zool. Fr.*, T. 47, pp. 495–504.

BOCQUET, C. & STOCK, J. H., 1957. Copépodes parasites d'Invertébrés des côtes de France. I. Sur deux genres de la famille de Clausidiidae commensaux de Mollusques: *Hersiliodes* Canu et *Conchyliurus* nov.gen. *Proc. Kon. nederl. Akad. Wetensch.*, Ser. C, Vol. 60, pp. 212–23. (Not seen.)

CANDEIAS, A., 1955. *Peniculus fistula* Nordm. on two new hosts *Trachurus trachurus* (L.) and *Trachurus picturatus* (Bowd.). *Notas Inst. Biol. marit.*, No. 8.

DAVENPORT, D., 1955. Specificity and behaviour in symbioses. *Quart. Rev. Biol.*, Vol. 30, pp. 29–46.

DELAMARE DEBOUTTEVILLE, C. & NUNES, L. P., 1951. Existence de 'formes biologiques' chez *Peniculus fistula* (Rudolphi) (Copepoda). *Vie et Milieu*, T. 2, pp. 448–58.

GIESBRECHT, W., 1899. Die Asterocheriden des Golfe von Neapel und des angrenzenden Meeres-Abschnitte. *Faun. u. Flor. Neapel*, Monogr. 25, 218 pp.

GOTTO, R. V., 1954. *Lichomolgides cuanensis* n.g., n.sp., an ascidicolous copepod occurring in *Trididemnum tenerum* (Verrill). *Parasitology*, Vol. 44, pp. 379–86.

GRAY, P., 1933. *Mycophilus rosovula* n.sp., a notodelphyoid copepod parasitic within *B.* (*Botrylloides*) *leachii* Sav. with a description of the nauplius and notes on the habits. *J. mar. biol. Ass. U.K.*, Vol. 18, pp. 523–8.

GURNEY, R., 1931. *British Fresh-water Copepoda. General Part and Calanoida*, Vol. 1, 238 pp. London: Ray Society.

—— 1933. Notes on some copepods from Plymouth. *J. mar. biol. Ass. U.K.*, Vol. 19, pp. 299–304.

HERDMAN, W. A. & SCOTT, A., 1896. Report on the investigations carried out in 1895 in connection with the Lancashire Sea-Fisheries Laboratory at University College, Liverpool. Section IV: Description of new and rare Copepoda (by A. SCOTT). *Proc. Lpool. biol. Soc.*, Vol. 10, pp. 103–77 (134–58).

HUMES, A. G., 1953. *Ostrincola gracilis* C. B. Wilson, a parasite of marine pelycopods in Louisiana (Copepoda Cyclopoida). *Tulane Stud. Zool.*, Vol. 1, pp. 99–107.

—— 1954. *Tisbe celata* n.sp., a Harpacticoid copepod from the mantle cavity of the Edible Mussel in New Brunswick. *J. Fish. Res. Bd Can.*, Vol. 11, pp. 816–26.

JOHNSON, M. W. & OLSON, J. B., 1948. The life history and biology of a marine Harpacticoid copepod, *Tisbe furcata* (Baird). *Biol. Bull., Woods Hole*, Vol. 95, pp. 320–32.

KIEFER, F., 1929. Crustacea Copepoda: II. Cyclopoida Gnathostoma. *Das Tierreich*, Bd. 53, 102 pp.

LANG, K., 1946*a*. A contribution to the question of the mouthparts of the Copepoda. *Ark. Zool.*, Bd. 38A, No. 5.

—— 1946*b*. Einige für die schwedische Fauna neue marine Cyclopoida Gnathostoma, nebst Bemerkungen über die Systematik der Unterfamilie Cyclopininae. *Ark. Zool.*, Bd. 38A, No. 6.

—— 1948. Copepoda 'Notodelphyoida' from the Swedish West Coast with an outline on the systematics of the Copepoda. *Ark. Zool.*, Bd. 40A, No. 14.

LEIGH-SHARPE, W. H., 1926. The Herpyllobiidae, a family of Copepoda parasitic on polynoid worms. *Parasitology*, Vol. 18, pp. 269–76.

—— 1933. Second list of parasitic Copepoda of Plymouth with a description of three new species. *Parasitology*, Vol. 25, pp. 113–18.

NICHOLLS, A. G., 1944. Littoral Copepoda from South Australia (II): Calanoida, Notodelphyoida, Monstrilloida and Caligoida. *Rec. S. Aust. Mus.*, Vol. 8, pp. 1–62.

NORDMANN, A., 1832. *Micrographische Beiträge zur Naturgeschichte der wirbellosen Thiere*, Heft 2, 150 pp. Berlin: Reimer.

NORMAN, A. M. & SCOTT, T., 1906. *Crustacea of Devon and Cornwall.* 232 pp. London: W. Wesley.

SARS, G. O., 1901. *An Account of the Crustacea of Norway*. Vol. 4, Copepoda, Calanoida. Parts 1 and 2. 28 pp. Bergen Museum.

—— 1913–18. *An Account of the Crustacea of Norway*, Vol. 6, Copepoda, Cyclopoida. 225 pp. Bergen.

SEWELL, R. B., 1949. The littoral and semi-parasitic Cyclopoida, the Monstrilloida and Notodelphyoida. *Sci. Rep. Murray Exped.*, Vol. 9, No. 2.

STOCK, J. H., 1953. *Bomolochus soleae* Claus, 1864 and *B. confusus* n.sp., two hitherto confounded parasitic copepods, with remarks on some other *Bomolochus* species. *Beaufortia*, No. 24.

WILSON, C. B., 1932. The Copepods of the Woods Hole Region Massachussetts. *Bull. U.S. nat. Mus.*, No. 158. 635 pp.

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ON THE ATTENUATION OF LIGHT IN THE SEA

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(Text-figs. 1 and 2)

Recently Jones & Wills (1956) have related the attenuation of light in the sea and in estuarine waters to the concentration of suspended solid matter. The attenuation was measured with an *in situ* hydrophotometer with a beam acceptance angle of approximately 3·20°. The concentration of suspended materials was determined by filtering in the case of natural samples or by adding known weights of kaolin or mud to tap water. In the discussion of their data and methods they have made application of the diffraction theory for opaque particles relatively large compared to the wave-length of light.

It seems worthwhile to extend the discussion in terms of electromagnetic theory for scattering of light by small relatively transparent spheres of the order in size of the wave-length of light. From an analysis of this type it may be possible to roughly estimate the predominant size of the material present in the water which was examined.

Fig. 1 (adapted from Burt, 1954) shows the theoretical extinction due to scattering as a function of particle size for uniform suspensions of small spherical particles. Extinction was computed for green light (550 mμ, approximately the centre of the visual response curve and thus corresponding to the centre of response curve of the hydrophotometer used by Jones & Wills) for 1 mg per litre suspensions of mineral material with a relative refractive index of 1·15 and a density of 2·65. The latest computations of Mie scattering for a refractive index of 1·15 were used (Pendorf, 1956). The solid lines are for light scattered in all directions, while the dashed line shows the theoretical extinction corrected to exclude light scattered into a cone of half angle 3° centred about the forward direction. The latter approximates the field of acceptance of the hydrophotometer.

Fig. 2, showing the experimental measurements of extinction v. concentration, is taken from Jones & Wills. Lines have been added which represent the theoretical relationship between extinction and concentration from the dashed line in Fig. 1. In making a rough comparison between the data and the theoretical results it should be kept in mind that a size range is present in each sample with particles of different density, shape and refractive index.

For the natural water samples taken from the Thames and the sea near Plymouth the predominant size range lies between 2·5 and 10 μ with a wide scatter between individual samples. There is a trend toward increase in particle size in the more turbid samples. The Thames Estuary samples tend to have larger particle sizes than those taken near Plymouth. The suspensions which were made up of Thames mud and tap water have predominate particle sizes near 3 μ, while the Kaolin suspensions probably have much smaller particles around 0·2 μ in diameter.

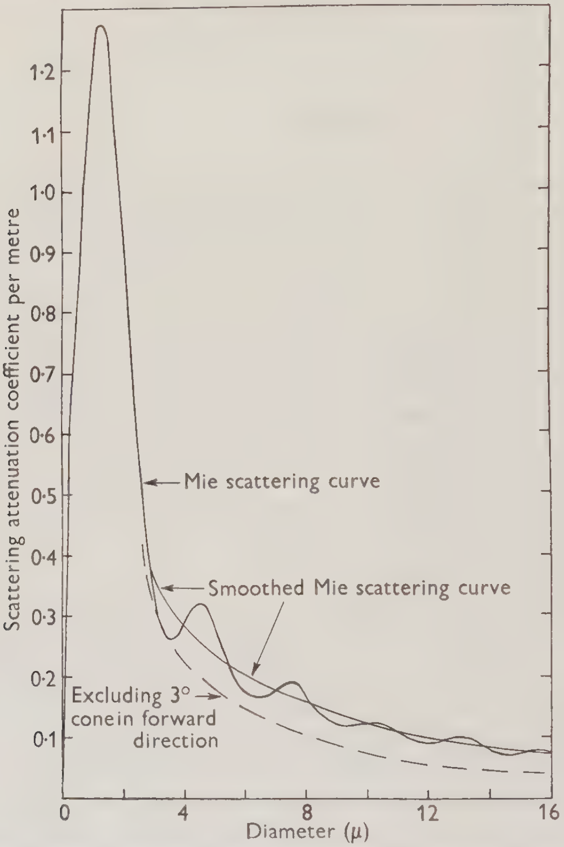

Fig. 1. Theoretical specific scattering for concentrations of suspended material of 1 mg per litre. Calculations are for green light (550 mμ) for material with a relative refractive index to water of 1·15.

The anomalous change in slope near the origin in the data for Kaolin and Thames mud suspensions may be due to fine material around 1·2 μ in diameter in suspension in the tap water which would displace the Kaolin and Thames mud suspension lines upward. Another possibility is that the dispersion may vary with the concentration at very low concentrations.

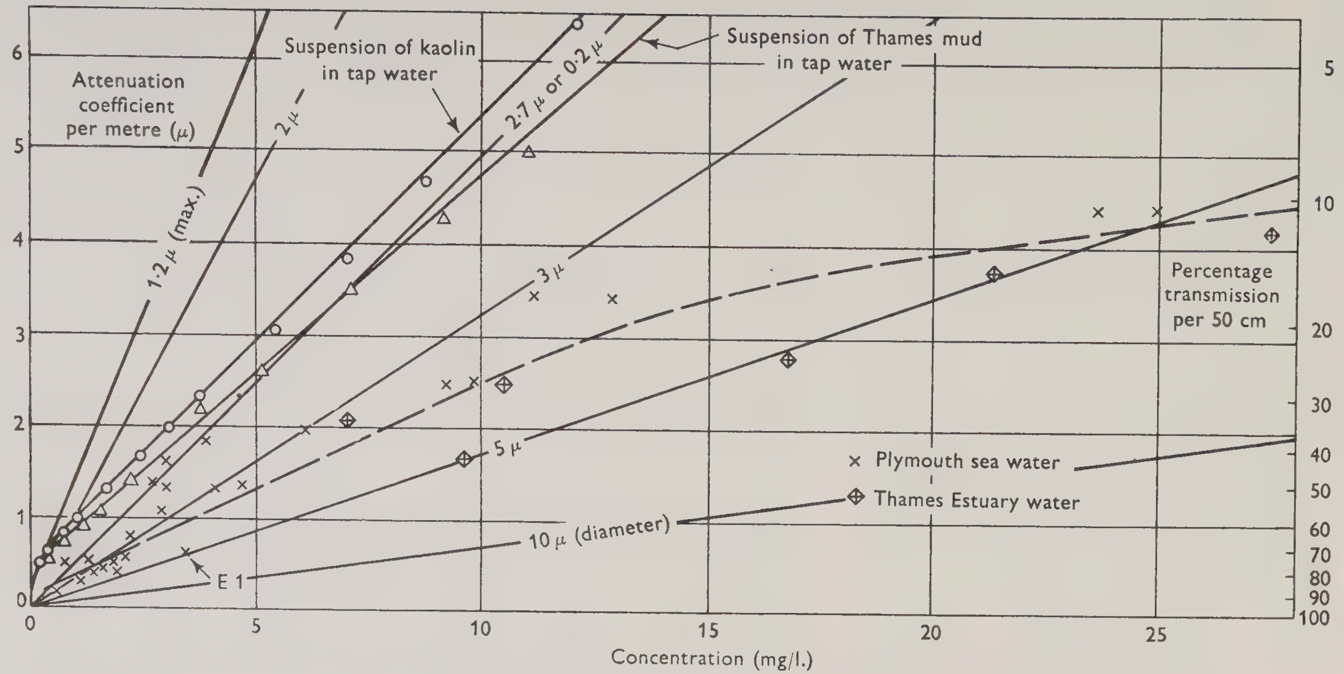

Fig. 2. Relation between attenuation coefficient per metre and concentration of suspended solid matter (from Jones & Wills, 1956). The solid lines have been added to show theoretical attenuation due to scattering by small particles with diameters of 0·2, 1·2, 2, 2·7, 3, 5 and 10 μ. E1 refers to a hydrographic station south of the Eddystone.

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REFERENCES

Burt, Wayne V., 1954. Specific scattering by uniform minerogenic suspensions. *Tellus*, Vol. 6, No. 3, pp. 229–31.

Jones, D. & Wills, M. S., 1956. The attenuation of light in sea and estuarine waters in relation to the concentration of suspended solid matter. *J. mar. biol. Ass. U.K.*, Vol. 35, No. 2, pp. 431–44.

Pendorf, R. B., 1956. New tables of Mie scattering functions for spherical particles. Part 6. Total Mie scattering coefficients for real refractive indices. *Geophysical Res. Papers*, No. 45, 98 pp. Air Force Cambridge Research Centre.

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NOTES ON OXYGEN SAMPLING ON THE FLADEN GROUND

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(Text-fig. 1)

For the years 1951–53 sampling of phosphate concentration has been used to describe the plant production on the Fladen Ground (Steele, 1956). Further studies in 1955–56 have compared the phosphate sampling with the ^{14}C technique (Steele, 1957). On most occasions samples were also taken for dissolved oxygen concentration and these notes discuss some attempts made to interpret those data. A set of oxygen profiles for Fladen are shown in the earlier paper.

There are four main causes of change in oxygen concentration; two physical, water mixing and surface exchange; and two biological, increase due to photosynthesis and decrease due to the breakdown of organic matter and to respiration. Except for surface exchange, these effects should have concomitant changes in the phosphate concentration and the main problems arise through the attempts to compare them.

When studying the phosphate data, the amount of vertical mixing was estimated from temperature profiles and its effect subtracted from the observed phosphate changes between two dates to leave what was called the 'biological change'. The same procedure can be carried out for the oxygen data except for the 0–20 m layer where an estimate of surface exchange is required before the biological change can be found. A fairly successful attempt to estimate exchange was made by Redfield (1948), using a formula which states in effect that the rate of exchange is proportional to the difference between the surface concentration and the equilibrium concentration. For Fladen, the best available oxygen data are those for 1953, when two stations were worked at fairly regular intervals and the computations of 'biological change' and surface exchange were made for these data. The values for oxygen solubility used in Redfield's formula were taken from the nomogram given by Richards & Corwin (1956), which is based on the data of Truesdale, Downing & Lowden (1955).

To compare these calculations with the similar ones for phosphate, a conversion factor is necessary, and one is given by Sverdrup, Johnson & Fleming (1942, p. 237). This depends on the phosphorus/carbon ratio found by chemical analysis of plankton and on an association of carbon and oxygen in the form CO_2. The numerical value in the usual units is

$$1\ \mu\text{g atom phosphorus} \equiv 2{\cdot}36\ \text{ml. oxygen}.$$

However, when this factor was used it was found that in the region below 40 m, where the 'biological changes' showed an increase for phosphate and a decrease for oxygen, the phosphate gain was equivalent to approximately two-thirds of the oxygen loss.

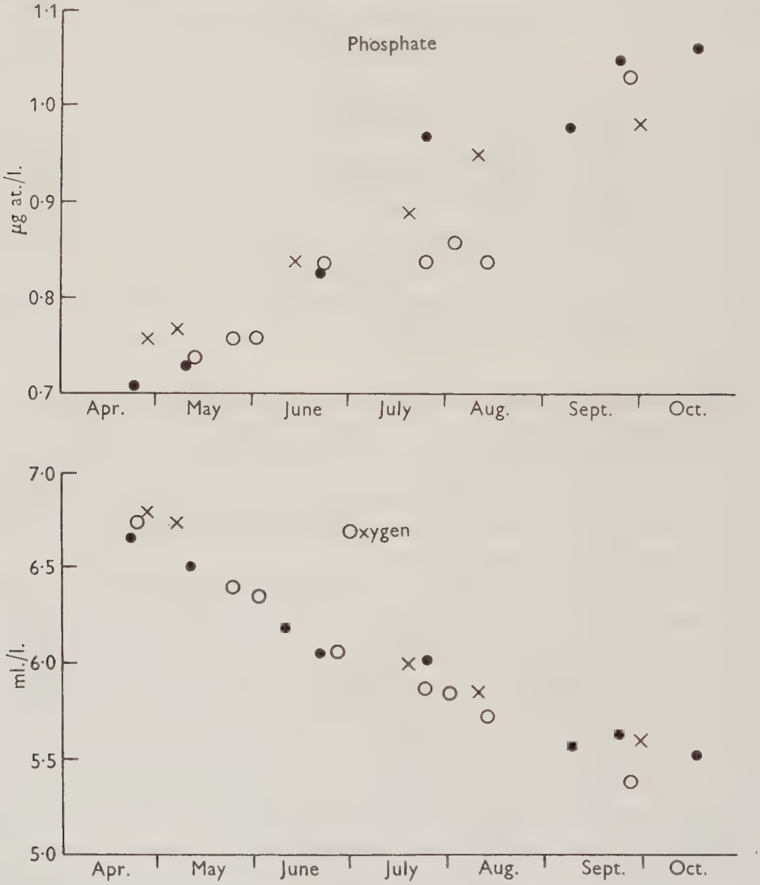

Fig. 1. The near-bottom concentrations of phosphate and oxygen for the years 1953 (●), 1955 (○) and 1956 (×).

To check this feature independently of the calculation, the oxygen and phosphate changes can be compared directly for the water below the thermocline which remains unmixed from April to October. In Fig. 1, the near-bottom concentrations are plotted for the years 1953, 1955 and 1956. There is considerable variability in the values, especially the phosphate, but the general rates of change seem to be consistent from year to year. These rates give a conversion factor of

$$1\ \mu\text{g atom phosphorus} \equiv 3{\cdot}87\ \text{ml. oxygen.}$$

The increase confirms the results of the calculations and the explanation probably lies in the fact that the basis of the oxygen/carbon comparison was a carbohydrate metabolism.

It has been shown that *Calanus finmarchicus* probably have a protein content of about 50% (Marshall, Nicholls & Orr, 1934) which would lead to a higher oxygen/phosphate ratio. In a full discussion of this problem Riley (1951) deduced that with a protein content of 68%, the metabolism of zooplankton and their bacterial oxidation would give a ratio approximately double the value given by Sverdrup *et al.* (1942). For phytoplankton it has usually been found that the photosynthetic quotient is nearly unity, which would entail a carbohydrate metabolism and so require the earlier conversion factor. However, Cramer & Myers (1948) have obtained a quotient of 1·47, and they believe that the previous values are due to a temporary shift to carbohydrate synthesis caused by the high light intensities required in the experiments (see also Ryther, 1956). Their results would lead to a conversion factor of 3·5 which is near the value given by the Fladen data. For these reasons, a conversion factor of about 3·87 may be applicable not only in the lower waters but also in the euphotic zone, and this value is used in the following comparison.

The biological changes in phosphate and oxygen are compared by converting the phosphate changes to oxygen units and then adding these to the calculated oxygen changes. What is left over will be termed the 'residual' thus:

$$\text{residual} = \text{oxygen change} + 3{\cdot}87 \text{ phosphate change.}$$

If the phosphate changes always corresponded to oxygen changes then the residuals would be zero except for the 0–20 m layer where they would be balanced by the surface exchange. Such agreement is not found and the values for the various factors are given in Table 1.

The first point to be noted is that the estimates of surface exchange are greater than the residuals for 0–20 m by a factor which varies from 1·05 to 8·8. Thus the formula does not appear to be successful in this case. Below 20 m the residuals are very variable, and all that can be said is that in general they are less than the oxygen changes; the few cases in which the oxygen changes have been accentuated, are printed in bold type.

Another problem concerning oxygen:phosphate ratios arises from results given by Riley (1956). By similar methods to those used for the Fladen data, he estimated the biological changes of oxygen and phosphate in Long Island Sound. He found that the over-all ratios for production were normal but the phosphate was absorbed at a lower level than the oxygen was produced. On Fladen, in the 20–30 m layer, this occurs to some extent in the spring but not in the summer (Table 1). However, it is the summer that is of most interest since at this time the phosphates show maximum production in the 20–30 m layer. Direct observations of this feature are available for one particular time interval, 21 June–24 July 1953, when there was very little mixing at or below

30 m. During this period the average changes at 30 m for four stations were: temperature, +0·25° C; phosphate, −0·19 μg at./l.; oxygen, +0·98 ml./l. In fact the oxygen increase is greater than that required by the phosphate decrease, so it would seem that the rate of production was at least that indicated by the phosphate data. This case may be peculiar due to the lack of vertical mixing, and later data using ^{14}C (Steele, 1957) do not support the phosphate results.

TABLE 1. ESTIMATES OF SURFACE EXCHANGE, 'BIOLOGICAL CHANGE' OF OXYGEN AND RESIDUALS FOR FLADEN IN 1953

	21. iv.–11. v.		11. v.–11. vi.		11–21. vi.		21. vi.–24. vii.	
Exchange...	—	3·36	—	6·36	—	1·52	—	3·64
(m)	Ox.	Res.	Ox.	Res.	Ox.	Res.	Ox.	Res.
0–20	1·62	−0·38	0·48	−1·37	−0·77	−1·02	−0·58	−1·40
20–30	−0·46	**−0·64**	−1·26	**−2·15**	−0·05	**−0·41**	0·96	0·11
30–40	−0·14	**−0·50**	−0·60	0·72	−0·32	−0·22	0·44	0·01
40–50	−0·38	0·01	−0·31	−0·23	−0·36	−0·03	0·04	**0·53**
50–60	−0·28	−0·05	−0·24	−0·13	−0·26	0·04	0	**0·39**
60–140	−0·86	−0·17	−1·74	0·46	−1·74	0·49	−0.04	**1·80**

	24. vii.–8. ix.		8–25. ix.		25. ix.–19. x.	
Exchange...	—	3·87	—	0·98	—	0·70
(m)	Ox.	Res.	Ox.	Res.	Ox.	Res.
0–20	−2·05	−2·30	−0·91	−0·93	0·49	−0·30
20–30	0·82	−0·26	0·71	0		
30–40	1·33	−1·31	−0·04	0·19	0·35	−0·44
40–50	−1·16	−0·08	−0·35	0·06	0·10	−0·24
50–60	−0·58	−0·35	−0·20	0·32	−0·02	0·06
60–140	−4·35	−2·86	0·28	**1·80**	−0·64	0·74

SUMMARY

A comparison is made of oxygen and phosphate data from the Fladen Ground. It is found that the conversion factor for oxygen:phosphorus given by Sverdrup *et al.* (1942) does not agree with the changes below the euphotic zone and an alternative factor is calculated. Problems concerning surface exchange and production are also discussed.

REFERENCES

CRAMER, M. & MYERS, J., 1948. Nitrate reduction and assimilation in *Chlorella*. *J. gen. Physiol.*, Vol. 32, pp. 93–102.

MARSHALL, S. M., NICHOLLS, A. G. & ORR, A. P., 1934. On the biology of *Calanus finmarchicus*. V. Seasonal distribution, size, weight and chemical composition in Loch Striven in 1933 and their relation to the phytoplankton. *J. mar. biol. Ass. U.K.*, Vol. 19, pp. 793–827.

REDFIELD, A. C., 1948. The exchange of oxygen across the sea surface. *J. mar. Res.*, Vol. 7, pp. 347–61.

RICHARDS, F. A. & CORWIN, N., 1956. Some oceanographic application of recent determinations of the solubility of oxygen in sea water. *Limnol. Oceanogr.*, Vol. 1, pp. 263–7.

RILEY, G. A., 1951. Oxygen, phosphate and nitrate in the Atlantic Ocean. *Bull. Bingham oceanogr. Coll.*, Vol. 13, Art. 1, 126 pp.

—— 1956. Oceanography of Long Island Sound, 1952–1954. IX. Production and utilization of organic matter. *Bull. Bingham oceanogr. Coll.*, Vol. 15, pp. 324–44.

RYTHER, J. H., 1956. The measurement of primary production. *Limnol. Oceanogr.*, Vol. 1, pp. 72–84.

STEELE, J. H., 1956. Plant production on the Fladen Ground. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 1–33.

—— 1957. A comparison of plant production estimates using ^{14}C and phosphate data. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 233–41.

SVERDRUP, H. U., JOHNSON, M. W. & FLEMING, R. H., 1942. *The Oceans; their Physics, Chemistry and General Biology*. 1087 pp. New York: Prentice-Hall.

TRUESDALE, G. A., DOWNING, A. L. & LOWDEN, G. F., 1955. The solubility of oxygen in pure water and sea water. *J. appl. Chem.*, Vol. 5, pp. 53–62.

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A COMPARISON OF PLANT PRODUCTION ESTIMATES USING ^{14}C AND PHOSPHATE DATA

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(Text-figs. 1–6)

In an earlier paper (Steele, 1956) estimates of the yearly cycle of plant production were given for a part of the northern North Sea known as the Fladen Ground. These estimates were made from a study of the changes in inorganic phosphate in relation to the effects of vertical mixing.

The method is indirect since several hypotheses have to be made relating phosphorus and carbon uptake by phytoplankton. The problems which these raise are most acute during the summer when the results show a low rate of production. Either of two factors, a high rate of regeneration of phosphorus to inorganic form in the euphotic zone, or the ability of plants to use organic phosphorus compounds, could make the production rate much greater.

For these reasons a check on the method was desirable and this has been done using the ^{14}C technique devised by Steeman Nielsen (1952) which gives directly a measure of the carbon uptake during a short period of time.

The main purpose of this paper is to show from data collected in 1955 and 1956 that there is general agreement between the two methods. This purpose is attained in so far as the mean daily production calculated from phosphates was 0·20 g C/m^2 between 1 June and 13 August 1955, while the three values found from the ^{14}C experiments during this period were 0·20, 0·21 and 0·15. The results are also used to continue the study of the changing vertical distribution of plants in terms of the effects of sinking and grazing.

It is a pleasure to thank Dr H. W. Harvey for his advice and Mr R. I. Currie of the National Institute of Oceanography for instruction in the details of the ^{14}C technique and for working up the samples collected in 1955.

METHODS

The technique for estimating ^{14}C uptake was essentially that described by Steeman Nielsen (1952). The only variation that was made in the method concerns the estimation of the activity of the ^{14}C solution from the self-absorption curve. It was found difficult to obtain satisfactory precipitates of very small quantities of barium carbonate, and the need for this was avoided by using the known form for the equation of the curve. This gives the activity at thickness T as

$$G(T) = G(O)\,\frac{1-e^{-\mu T}}{\mu T},$$

where μ is a constant and $G(O)$ is the value required (Calvin *et al.*, 1949, p. 30). A curve is fitted to the mean values at three thicknesses; the goodness of the fit acting as a check. Fig. 1 gives the curve for the ampoules used in 1956.

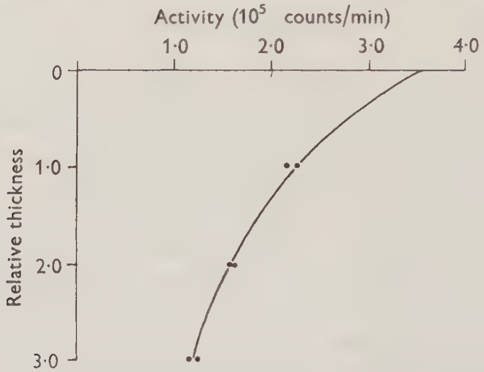

Fig. 1. Self-absorption curve for the ampoules used in 1956.

The bottles used in the experiments were of approximately 170 c.c. capacity. They were filled in pairs from the following depths: 1, 10, 20, 30, 40 and 50 m. The bottles were lowered to the depths from which the water came and left there for the whole (1955) or half (1956) of the period of daylight.

The methods of estimating production from the phosphate data, chlorophyll concentration and zooplankton dry weight have already been described (Steele, 1956).

ESTIMATION OF CARBON ASSIMILATION

In his experiments, Steeman Nielsen collected water samples from different depths and exposed them in a water bath to the same light intensity for 4 h. In converting the measures of ^{14}C uptake to values of carbon assimilation he allowed 6% for isotope factor and 4% for loss due to respiration giving a total addition of 10%.

When samples are exposed at different depths the effect of respiration will be proportionally greater in the deeper water where there is a low rate of photosynthesis. The importance of this effect will depend on the extent to which the ^{14}C is available for respiration during the period of the experiment. Since there is not yet sufficient evidence to estimate this effect, the values for carbon assimilation were calculated by Steeman Nielsen's method. These values are shown in Figs. 2 and 3, together with the profiles of population, estimated from the chlorophyll concentrations.

It is generally thought that photosynthesis varies directly with the light intensity except near the surface where there is some inhibition (Jenkins, 1937; Steeman Nielsen, 1952). To study the present data from this point of view, Secchi disc readings (Table 2) were used to estimate the extinction coefficient, K, defined by the rough conversion factor of Poole & Atkins (1929) as

$$K = \frac{1{\cdot}7}{\text{S.D. reading}}.$$

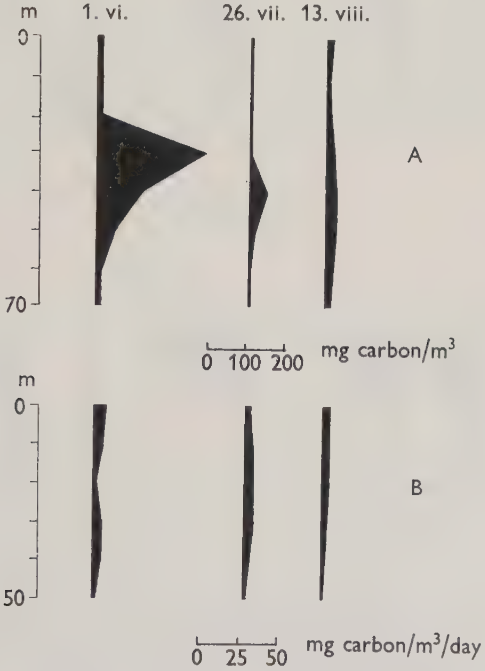

Fig. 2. Profiles of (A) population and (B) rate of production on the Fladen Ground during 1955.

Assuming that the light which is effective for photosynthesis decreases as $\exp(-KZ)$, where Z is the depth in metres, then the light intensity relative to the surface can be found. Since no measurements were made of the absolute light intensity at the surface, the values for each date are not directly comparable. On this basis the values for C shown in Table 1 were calculated where

$$C = \frac{\text{carbon assimilation/day}}{\text{population} \times \text{rel. light intensity}}.$$

Thus *C* provides an index for the increase in cellular carbon in terms of the quantity of chlorophyll present multiplied by a rough measure of the relative light energy at each depth.

There is considerable variation in the values of *C*, but this is not large in terms of the range of values of both assimilation and light intensity which can vary by a factor of several hundreds between surface and 50 m. The expected

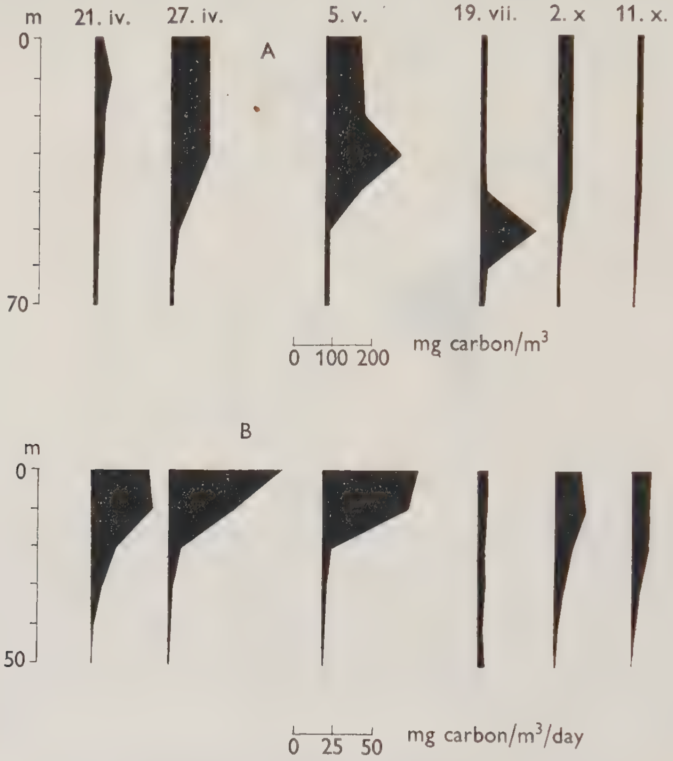

Fig. 3. Profiles of (A) population, and (B) rate of production on the Fladen Ground during 1956.

TABLE 1. VALUES OF *C* DURING 1955 AND 1956 (SEE TEXT)

	Depth (m)					
Date	1	10	20	30	40	50
1. vi. 55	0·5	1·3	1·0	0·5	3·0	1·5
26. vii. 55	0·5	2·1	3·8	12·0	3·2	5·0
13. viii. 55	0·2	0·8	1·6	1·0	0·8	0·5
21. iv. 56	1·8	2·8	5·8	8·6	6·6	20·0
27. iv. 56	0·8	2·4	2·4	2·0	16·0	22·0
5. v. 56	0·8	3·4	1·8	1·4	2·0	—
19. vii. 56	—	1·4	2·8	8·8	10·0	5·2
2. x. 56	0·5	1·4	2·0	2·8	2·4	3·1
11. x. 56	0·7	2·0	5·8	7·6	11·2	16·4

decrease in C near the surface is found and it occurs also to some extent at 10 m. At 40 and 50 m the values are more variable, but in general they tend to be larger. It is at these depths, however, that lower values of C would be expected if much of the ^{14}C were being lost through respiration. On this rather tenuous basis it would appear that ^{14}C estimates more nearly represent total photosynthesis rather than net assimilation.

Some idea of the magnitude of the respiration loss can be obtained from the formula given by Riley, Stommel & Bumpus (1949),

$$r_T = 0{\cdot}0175 \exp (0{\cdot}069\, T),$$

where r_T is the respiratory coefficient in g C/day/g phytoplankton carbon at temperature T. In Table 2 the ratios of respiration to production are given as the average for the layer 0–50 m. Only one value exceeds one third and the mean is about 0·22 which can be considered a reasonable general value (Steeman Nielsen, 1952). There is a considerable fluctuation in the figures, and for this reason no allowance has been made for respiration in the following discussion.

TABLE 2. OBSERVATIONS ASSOCIATED WITH ^{14}C EXPERIMENTS (SEE TEXT)

	1955			1956					
	1. vi.	26. vii.	13. viii.	21. iv.	27. iv.	5. v.	19. vii.	2. x.	11. x.
Secchi disc reading (m)	15·5	19·0	21·0	15·0	10·0	10·0	17·0	18·0	14·5
Respiration/production	0·65	0·15	0·33	0·04	0·11	0·19	0·21	0·18	0·09
Mean dry weight of zooplankton (g C/m^2)	2·2	1·4	2·3	0·7	2·1	2·7	4·0	0·6	0·4

COMPARISON OF PRODUCTION ESTIMATES

The general comparison of the estimates of production given by ^{14}C and by phosphate change is shown in Fig. 4. A detailed comparison is difficult since the ^{14}C uptake measures production rate on a given day, whereas the phosphate data give a value for production between two dates. The main point is that there is no large and systematic discrepancy between the two sets of data. The ^{14}C values are on the average slightly less than the phosphate values and the difference would be accentuated if respiration were taken into account. This is important for, on this basis, there is no evidence that the phosphate estimates are fallaciously low due to regeneration or to the use of organic phosphorus compounds by the phytoplankton. To this extent the phosphate method is validated.

Given this general agreement between phosphate and ^{14}C estimates one main difference must be noted; this is found in the depth distribution of production. The most suitable examples are the four occasions when two ^{14}C experiments were made within a short time interval. The phosphate profiles for these intervals are shown in Fig. 5, and it can be seen that they differ from the relevant ^{14}C profiles of Figs. 2 and 3.

For the final interval, 2–11 October 1956, the difference can be explained by the fact that the water column is homogeneous from 0 to 40 m. This means that, although the production rate at any moment has the depth distribution shown by the ^{14}C data, mixing spreads this production evenly through the homogeneous layer so that, effectively, production is constant in this depth range. However, this type of explanation is not sufficient to account

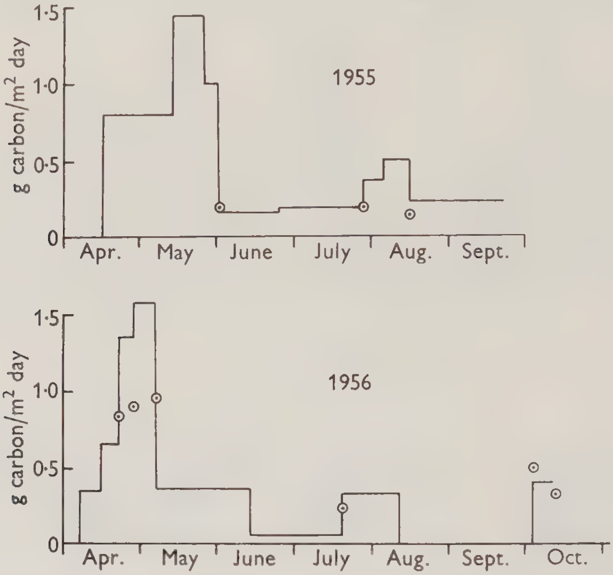

Fig. 4. Histograms of production for the years 1955 and 1956 derived from the phosphate data. The circles show the rates of production estimated by the ^{14}C method.

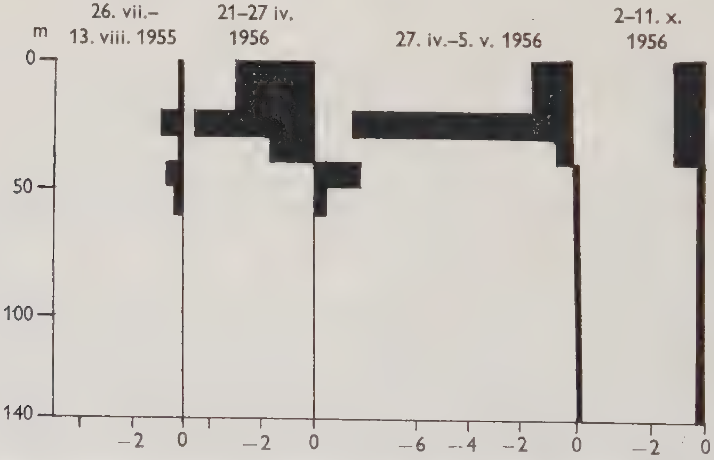

Fig. 5. Some profiles of 'biological change' of phosphate on Fladen in $10^2 \times \mu$g at./cm² day.

for the fact that maximum phosphate consumption in the summer seems to occur between 20 and 30 m (Steele, 1956). Oxygen data appear to support the phosphate results (Steele, 1957) but, although the anomaly remains, it seems probable that the ^{14}C values are more trustworthy.

SINKING AND FILTERING RATES

In the earlier paper (Steele, 1956) an attempt was made to explain variations in plant population throughout 1953 in terms of production, sinking, grazing and vertical mixing. The first step was to calculate sinking rates of phytoplankton and filtering rates of zooplankton from the mean changes in and below the euphotic zone during the interval between two sampling dates. Using these values and the population profile at the beginning of the interval, a profile was derived for the end of the interval which could be compared with the observed profile. As a continuation of this work the four intervals discussed in the previous section were analysed in the same way using the ^{14}C estimates of production. The values for dry weight of zooplankton used in the equations are given in Table 2.

TABLE 3. SINKING AND FILTERING RATES

	1955	1956		
	26. vii.–13. viii.	21–27. iv.	27. iv.–5. v.	2–11. x.
Sinking rate (m/day)	2·2	3·3	1·9	6·0
Filtering rate (m^3/day/g carbon)	5·8	7·7	7·7	78·0

Table 3 gives the estimated sinking and filtering rates. The values for the last interval are excessively high and no satisfactory explanation can be given. The remaining values are comparable with those of 1953. In particular, the higher filtering rate in the spring agrees with the conclusions made in the earlier paper.

Fig. 6 shows the comparison of observed and calculated profiles for the first three intervals. The agreement between them is not very good; in particular the sharp increase at 30 m on 5 May 1956 cannot be explained. The use of the phosphate estimates of production does not give any appreciable improvement.

CONCLUSIONS

As so often happens in this type of work the results are not completely consistent. Hypotheses are partly supported, partly disproved, and further data alter previous conclusions. Such difficulties have arisen in these attempts to explain in detail the changing vertical distribution of production and of population.

In three out of the four cases considered, a broad division of the water column into the euphotic zone and the region below leads to estimates of sinking and filtering rates which are in good agreement with previous values.

The profiles deduced from these rates are not always satisfactory, and the question remains whether this defect is due to some combination of sampling errors, inadequacy of approximating over periods of weeks, and arithmetical artifacts; or are the hypotheses themselves insufficient to explain such detail? One obvious conclusion is that much further study is required on these basic problems.

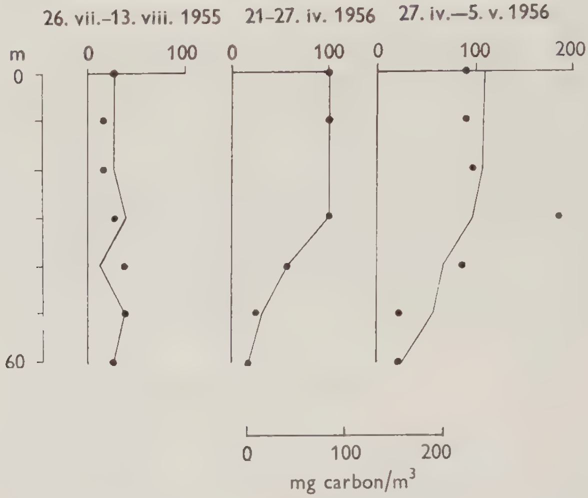

Fig. 6. Comparison of observed and calculated population profiles.

A rough analysis of the depth distributions of ^{14}C uptake gives some evidence that these agree with the accepted relations of photosynthesis and light intensity. For this reason these distributions are more acceptable than those given by the phosphate data.

There remains the fact that as measures of production below a given area of sea surface the two methods are in good agreement. Differences are to be expected since there will be day-to-day variations in carbon uptake due to changes in weather, vertical mixing, etc. From this point of view phosphate estimates are valuable since they measure production during an interval of weeks. Further, because of the way they are derived, phosphate estimates display the relations between production and such features of the environment as vertical mixing and nutrient deficiency. For these reasons the two methods may be regarded as complementary.

SUMMARY

Organic production in the sea can be measured by studying the changes in phosphate throughout the water column. Results obtained by this method are compared with estimates of carbon uptake using ^{14}C made on the Fladen

Ground during 1955 and 1956. There is good general agreement between the two methods but some differences in the vertical distribution.

The results are also used to calculate filtering rates of phytoplankton and sinking rates of zooplankton, and to compare observed and predicted vertical distributions of chlorophyll.

REFERENCES

CALVIN, M., *et al.*, 1949. *Isotopic Carbon.* 376 pp. New York: Wiley.

JENKINS, P. M., 1937. Oxygen production by the diatom *Coscinodiscus excentricus* Ehr. in relation to submarine illumination in the English Channel. *J. mar. biol. Ass. U.K.*, Vol. 22, pp. 301–43.

POOLE, H. H. & ATKINS, W. R. G., 1929. Photo-electric measurements of submarine illumination throughout the year. *J. mar. biol. Ass. U.K.*, Vol. 20, pp. 1–27.

RILEY, G. A., STOMMEL, H. & BUMPUS, D. F., 1949. Quantitative ecology of the plankton of the western North Atlantic. *Bull. Bingham oceanogr. Coll.*, Vol. 12, Art. 3, 169 pp.

STEELE, J. H., 1956. Plant production on the Fladen Ground. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 1–33.

—— 1957. Notes on oxygen sampling on the Fladen Ground. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 227–31.

STEEMAN NIELSEN, E., 1952. The use of radio-active carbon (C^{14}) for measuring organic production in the sea. *J. Cons. int. Explor. Mer.*, Vol. 18, pp. 117–40.

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THE LARVAE OF SOME MONOGENETIC TREMATODE PARASITES OF PLYMOUTH FISHES

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(Text-figs. 1–28)

The Order Monogenea of the Class Trematoda contains (Sproston, 1946) upwards of 679 species, but in only twenty-four of these species has a larval form been described. Of these larvae, fourteen belong to adults that parasitize fresh-water fishes, amphibians or reptiles, and ten to adults that parasitize marine fishes. *Udonella caligorum* is known (Sproston, 1946) not to have a larval stage in its life history. In the present study, accounts will be given of eleven hitherto undescribed larvae which belong to adults that parasitize marine fishes at Plymouth, and which represent seven of the eighteen families (Sproston, 1946) of the Monogenea.

For the most part the literature on larval monogeneans consists of isolated studies of individual species, and these have been listed by Frankland (1955), but the descriptions of four new larvae by Euzet (1955) appeared too late for inclusion in Frankland's list. More general observations on monogenean life cycles have been made by Stunkard (1937) and Baer (1951), and Alvey (1936) has speculated upon the phylogenetic significance of monogenean larvae.

Previous accounts of monogenean larvae have included but scant reference to culture techniques, presumably because the methods adopted have been simple and successful; in my hands, however, many attempts to rear larvae have been unsuccessful, and so some details of those procedures which have yielded successful cultures are included. At the same time it must be pointed out that the main object of the present study was to obtain specimens of monogenean larvae for morphological investigation, and properly designed experiments to determine factors influencing embryonic development were not attempted.

In addition to the studies made upon the eleven previously undescribed larvae, observations were made upon the larvae of *Diclidophora luscae* and *Polystoma integerrimum* in order to facilitate comparisons and to provide practice in techniques.

In past accounts of monogenean larvae it has been customary to describe the egg capsules, but the variations in the form of the capsules of the species studied here appear likely to be related more to variations in habits of oviposition than to larval characters, and so it is intended later to study the egg capsules in relation to their respective adults. However, it seems worth while to record now that the thirteen species of monogeneans that I have studied, together with those in previous descriptions of larvae, all have operculate capsules. It is remarkable therefore that Dawes (1946, p. 13) should have stated that in Monogenea, operculate eggs are sufficiently rare to make this character of general diagnostic value in distinguishing between Monogenea (non-operculate eggs) and Digenea (operculate eggs).

MATERIAL AND TECHNIQUES

Specimens of adult trematodes were obtained from fishes at Plymouth in July and August, some in 1954 and 1955, but mainly in 1956. The gills of the fish hosts were examined in a manner described previously (Llewellyn, 1956), and in addition trematodes were obtained from the skin of the Common Sole and the Cuckoo Ray. A list of the parasites studied, named and classified according to Sproston (1946), is included in Table 1.

TABLE 1. CLASSIFIED LIST OF THE PARASITES STUDIED

		Host	
Monopisthocotylea			
Gyrodactyloidea			
Dactylogyridae	*Diplectanum aequans*	*Morone labrax*	Gills
Capsaloidea			
Capsalidae	*Entobdella soleae*	*Solea solea*	Skin
Acanthocotyloidea			
Acanthocotylidae	*Acanthocotyle lobianchi*	*Raja clavata*	Skin
Polyopisthocotylea			
Avielloidea			
Polystomatoidea			
Polystomatidae	**Polystoma integerrimum*	*Rana temporaria*	Bladder
Hexabothriidae	*Rajonchocotyle emarginata*	*Raja clavata*	Gills
Diclidophoroidea			
Discocotylidae	*Plectanocotyle gurnardi*	*Trigla cuculus*	Gills
	Anthocotyle merluccii	*Merluccius merluccius*	Gills
Microcotylidae	*Gastrocotyle trachuri*	*Trachurus trachurus*	Gills
	Pseudaxine trachuri	*Trachurus trachurus*	Gills
	†Unidentified microcotylid species	*Trachurus trachurus*	Gills
	Microcotyle labracis	*Morone labrax*	Gills
Diclidophoridae	*Diclidophora merlangi*	*Gadus merlangus*	Gills
	**Diclidophora luscae*	*Gadus luscus*	Gills

* Described by previous authors, but studied from fresh material in the present investigation.

† This is the same trematode as referred to previously (Llewellyn, 1956, p. 117); a description of the adult will be published later.

Adult trematodes were transferred as soon as possible to dishes of fresh sea water and rinsed free of mucus with jets of sea water from a pipette. Even specimens that appeared moribund usually became active after this treatment, although it was sometimes several hours before egg-laying was resumed. The trematodes were rinsed in several changes of Berkefeld-filtered sea water and then placed at the various controlled temperatures that from time to time became available. As stated previously, it was not possible to conduct properly designed experiments on the factors influencing the rate of egg-capsule production and the period of embryonic development, but the following general observations on about 480 adults and about 2600 capsules may be of use to future workers.

It seems likely that although adult parasites survive for at least 2 or 3 weeks, and probably longer at temperatures of 3–7° C, very few or no egg capsules are produced below about 8° C. At 13° C, which is approximately the temperature of the bottom water in the localities from which the hosts were trawled, the rate of capsule production per parasite per hour varies according to species between about 1·0 and 0·5 on the first day or two, falling to about 0·25 on the third day, and ceasing altogether on the fourth day excepting in *Entobdella*, which continues to lay at gradually decreasing rates for another 2 or 3 days. At 18° C the rate increases to about 3 or 4 capsules per hour for the first 12 h, but the parasites do not survive longer than about 24 h. At 20° C in most species there was no egg production, and the parasites died within about 12 h, but *Plectanocotyle*, *Pseudaxine*, *Gastrocotyle*, *Microcotyle* and *Diplectanum* were able to lay about 0·5 per hour before dying.

An attempt was made to collect eggs from parasites still attached to a living host by keeping isolated specimens of living *Trachurus trachurus* in small tanks, but a collection of only fifty-seven capsules in 7 h from a host subsequently found to be harbouring thirty specimens of *Gastrocotyle trachuri* was disappointing, and collecting from isolated parasites was thought to be more profitable.

Culture dishes were examined daily with a stereomicroscope, and egg capsules were transferred by means of a pipette to dishes of fresh, filtered sea water. Dishes of various shapes and sizes were tried, and eventually it was found that covered Petri dishes of 4 cm diameter and 2 cm depth provided the most acceptable compromise between the conflicting desiderata of a small volume of water to facilitate searching for and capturing larvae with a pipette, and a large volume to minimize the frequently fatal osmotic effects due to evaporation from the sea water. The use of filtered sea water, combined with incubation in darkness, helped to restrict contamination of the cultures, for while the presence of extraneous organisms appeared to have little effect upon actual embryonic development, conditions for observation were made more difficult. The chief disadvantage of contamination, however, was the damaging attack by ciliates upon larvae during the period of their actual emergence from the capsule.

Little or no embryonic development took place in any species at temperatures of 8° C and below. At 13° C the period of embryonic development in *Plectanocotyle* was found to be something between 21 and 30 days, precise determination being complicated by the habit of the adult of accumulating the capsules in the uterus for 3 or 4 days before laying, and thus the exact age of the capsules at the commencement of incubation was not known. Embryos of *Anthocotyle*, *Gastrocotyle* and *Diclidophora merlangi* all failed to complete development at 13° C in the 28-day period available to me for observation. At 18° C *Plectanocotyle* hatched after 13–16 days' incubation, and *Anthocotyle* after 21 days. At 20° C the periods in days of embryonic development were as follows: *Diplectanum*, 5; *Plectanocotyle*, 8–11; 'microcotylid species', 10; *Acanthocotyle*, 12; *Entobdella*, *Microcotyle labracis*, *Gastrocotyle* and *Pseudaxine*, 14–16; *Rajonchocotyle*, 25; and *Diclidophora merlangi*, 27.

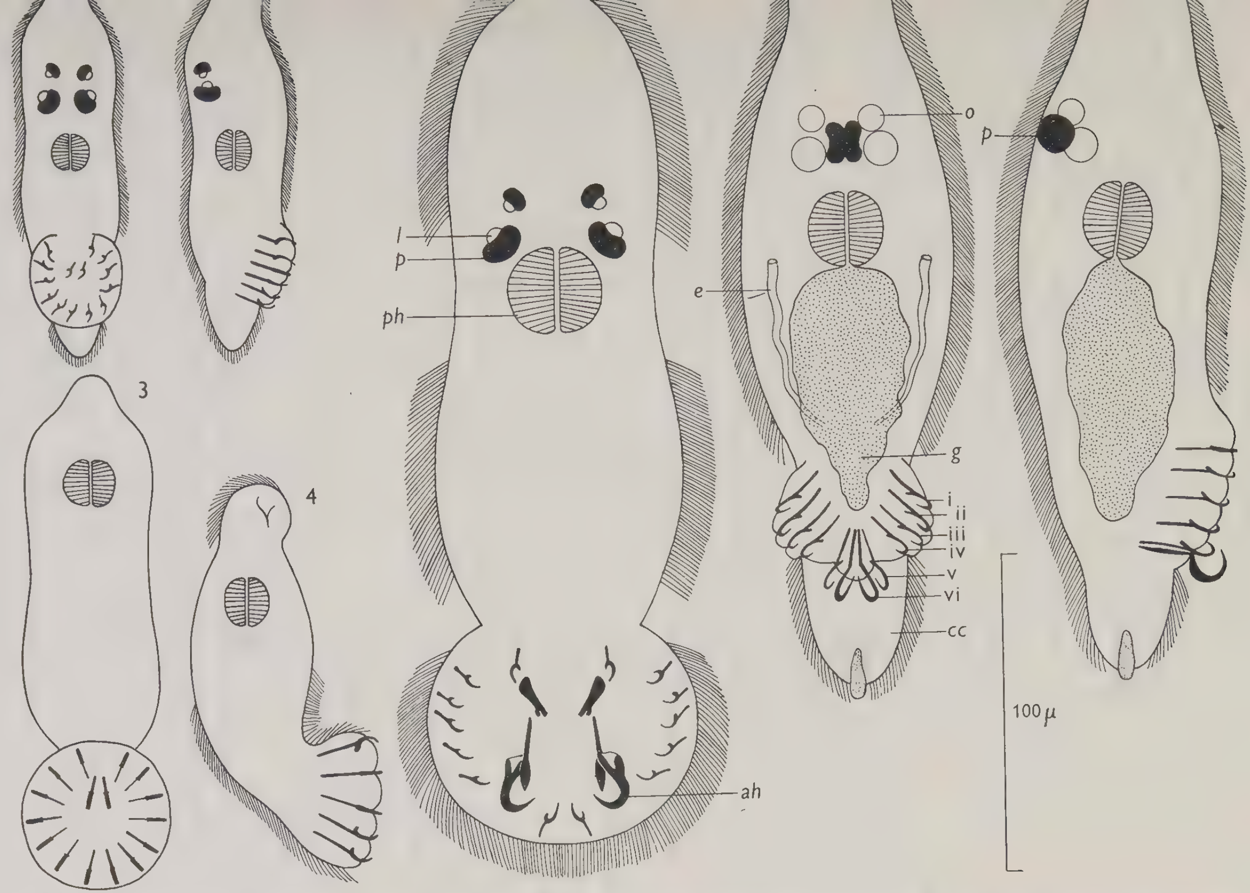
Hatching in all species appeared to be brought about entirely by repeated pressure against the operculum by muscular extension of the larvae as described by Frankland (1955) for *Diclidophora denticulata*, and in no case was there present a 'viscous cushion' mechanism as has been described in *Fasciola hepatica* by Rowan (1956).

From time to time it was found convenient to delay hatching by storing capsules containing active larvae at a temperature of about 4° C. Such specimens could subsequently be induced to hatch by returning them to a temperature of about 20° C, following which hatching usually took place in the course of some 6–12 h.

Free-living larvae and active larvae still enclosed in their capsules were examined under the microscope by mounting them on slides in sea water under cover-glasses supported by petroleum jelly in the manner generally practised for the study of digenean larvae. These fresh preparations, pressed to the required degree by the withdrawal of sea water with filter paper, were found to be much more useful than more permanent preparations, the best of which were made by mounting specimens either directly in Farrant's Medium, or, after fixation in formaldehyde and staining in borax carmine, in Canada Balsam. With temporary preparations, an apochromatic water-immersion objective was found more suitable than an oil-immersion objective since there was less tendency for the cover-glass to be moved with the objective during focusing. Extensive use of photography was made in recording the shapes of the larvae, and Figs. 1–28 were prepared from such photographs.

DESCRIPTIONS OF THE LARVAE (Figs. 1–28)

The larvae of the monogeneans studied are all cylindrical or ovoid organisms between 100 and 300 μ long, and between 30 and 100 μ in diameter. Although belonging to the 'flatworm' phylum, the degree of dorso-ventral flattening in these larvae is at most only very slight (compare Fig. 1 *a* with *b*, and Fig. 5 *a* with *b*). The anterior end of the body is tapered to form a conical region that may be marked off from the rest of the body by a shoulder region, and the posterior end is similarly tapered (*Diplectanum*, Fig. 1 *a*, *b*, and the diclidophoroideans, Fig. 5 *a*, *b*), or ends in a terminal adhesive organ (*Entobdella*, *Acanthocotyle*, *Rajonchocotyle*, Figs. 2–4 respectively).



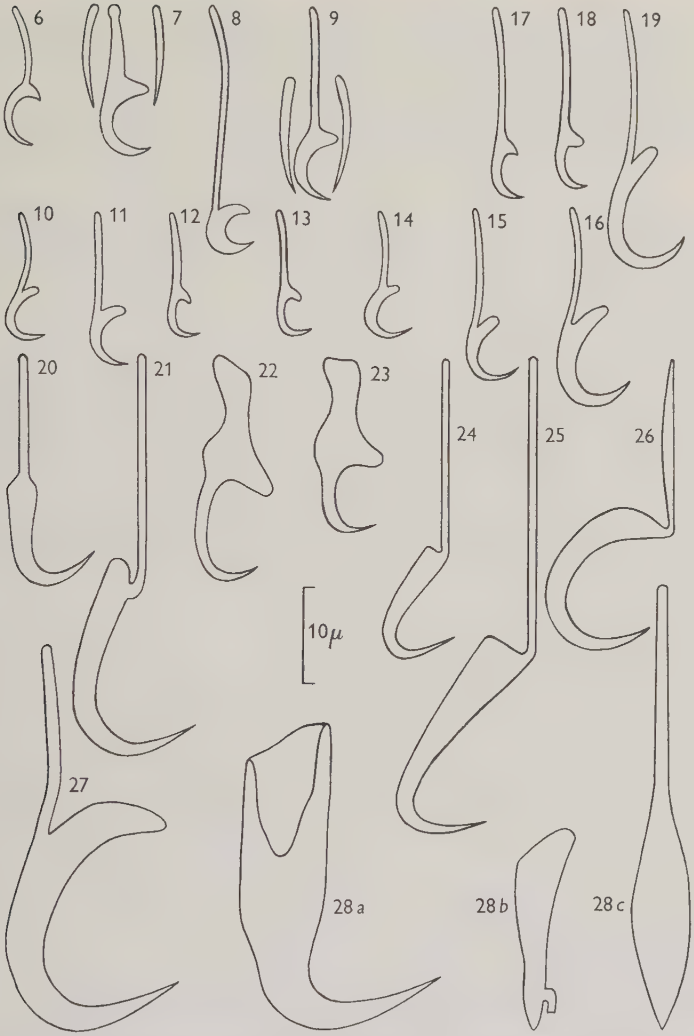
Figs. 1–5. Monogenean larvae, all drawn to same scale (ventral view unless otherwise stated). Fig. 1*a*, *Diplectanum aequans*. Fig. 1*b*, *D. aequans* (side view). Fig. 2, *Entobdella soleae*. Fig. 3, *Acanthocotyle lobianchi*. Fig. 4, *Rajonchocotyle emarginata* (side view). Fig. 5*a*, *b*, schematized larva of the superfamily Diclidophoroidea (Fig. 5*b* side view). *ah*, primordia of hooks of adult; *cc*, ciliated cone; *e*, excretory canal; *g*, gut; *l*, lens of eye; *m*, mouth; *o*, oil droplet; *p*, pigment cup of eye; *ph*, pharynx; i–iv, lateral hooks; v, postero-lateral hook; vi, posterior hook.

In all larvae except *Acanthocotyle* the body is ciliated, the extent of the ciliated areas varying in the different species.

In *Rajonchocotyle* and the diclidophoroideans, the mouth is situated anteriorly and terminally; the pharynx in these animals lies between a third and a half of the body length from the anterior end, and so there is presumably a very long pre-pharynx. I was unable to identify a mouth in *Diplectanum*, *Entobdella*, or *Acanthocotyle*, but a pharynx is readily distinguishable. It is possible that in these larvae, as in the larva of *Benedenia melleni* described by Jahn & Kuhn (1932), there is no pre-pharynx, the mouth leading directly into the pharynx. Posterior to the pharynx a simple gut is recognizable in most species as a region of densely massed refractile droplets that readily take up Sudan dyes and blacken with osmium tetroxide, and which are soluble in xylene. These oil droplets are not confined to the gut region; some are scattered throughout the body, with aggregations at the extreme anterior end, and, especially when eyes are present, near to these organs.

Eyes are present in all the larvae except *Acanthocotyle*, *Rajonchocotyle* and *Diclidophora merlangi*. They are situated dorsally at about one-third of the body length from the anterior end, and may consist of a pair of pigment cups with the openings directed laterally (Diclidophoroidea, Fig. 5*a*, *b*), or of two pairs of pigment cups (*Diplectanum*, Fig. 1*a*, *b*; *Entobdella*, Fig. 2). When two pairs of eyes are present, those of the anterior pair are smaller and are directed postero-laterally, and those of the posterior pair are larger and directed antero-laterally. In *Diplectanum* and *Entobdella* each pigment cup is provided with a spherical lens, but the diclidophoroideans do not have permanent lenses. It is possible, however, that the large conspicuous oil drops associated with the eyes of diclidophoroideans (Fig. 5*a*, *b*) may function as temporary lenses. In this connexion it is perhaps of significance that the eyes are retained in the adult *Diplectanum* and *Entobdella*, but disappear in adult diclidophoroideans.

At the posterior end of all the larvae is an adhesive organ which is terminal in *Entobdella*, *Acanthocotyle* and *Rajonchocotyle*, but ventral (i.e. succeeded posteriorly by some other part of the body) in *Diplectanum* and all the diclidophoroideans. The adhesive organ always bears hooks, the arrangement, shapes, and sizes of which are characteristic of the species. In *Entobdella* and *Rajonchocotyle* each larval hook is provided with a pair of accessory pieces (Figs. 7, 9). Such structures in *Polystoma integerrimum* have been described by Halkin (1901) as 'ogives', and their appearance in diagrams has given rise to the erroneous impression (e.g. Dawes, 1946) that the hooks of *Polystoma* are 'arrow-headed'. Since the presence of ogives has been described in various diclidophoroideans by Gallien (1934), Gallien & Le Calvez (1947), and Euzet (1955), great care was taken in searching for such structures in the diclidophoroideans studied in the present investigation. Thoroughly flattened specimens, mounted in sea water, glycerin, Farrant's Medium, or Canada



Figs. 6–28. Hooks of larval monogeneans, all drawn to same scale. Fig. 6, *Diplectanum aequans*. Fig. 7, *Entobdella soleae* (with ogives). Fig. 8, *Acanthocotyle lobianchi*. Fig. 9, *Rajonchocotyle emarginata* (with ogives). Figs. 10–16, typical lateral hooks of diclidophoroidean larvae. Fig. 10, *Plectanocotyle gurnardi*. Fig. 11, *Anthocotyle merluccii*. Fig. 12, *Gastrocotyle trachuri*. Fig. 13, *Pseudaxine trachuri*. Fig. 14, unidentified microcotylid species. Fig. 15, *Microcotyle labracis*. Fig. 16, *Diclidophora merlangi*. Figs. 17–19, postero-lateral hooks of diclidophoroideans. Fig. 17, *Gastrocotyle trachuri*. Fig. 18, *Pseudaxine trachuri*. Fig. 19, *Diclidophora merlangi*. Figs. 20–26, posterior hooks of diclidophoroideans. Fig. 20, *Plectanocotyle gurnardi*. Fig. 21, *Anthocotyle merluccii*. Fig. 22, *Gastrocotyle trachuri*. Fig. 23, *Pseudaxine trachuri*. Fig. 24, unidentified microcotylid species. Fig. 25, *Microcotyle labracis*. Fig. 26, *Diclidophora merlangi*. Figs. 27–28, primordia of adult hooks present in larva at time of hatching. Fig. 27, *Microcotyle labracis*. Fig. 28 *a*, *b*, *c*, 3rd, 1st and 2nd hooks respectively of *Entobdella soleae*.

Balsam, were examined with an oil-immersion objective of 1·40 N.A. Especial care was taken in examining the regions adjacent to the curved parts of the hooks, since parts of the hook often curve out of focus and reappear as apparently discrete structures. I was unable to find any ogives in any of the seven species of the Diclidophoroidea studied here, or in *Diclidophora luscae*, a species in which Gallien (1934) had previously described ogives as being present.

I have observed flame cells and a pair of longitudinal excretory canals opening by pores situated one on each side of the pharynx in *Pseudaxine* and some other diclidophoroideans, but I have not made a detailed investigation of the excretory systems.

Diplectanum aequans (Fig. 1 *a*, *b*)

Length 100–130 μ. Body, excepting posterior adhesive organ, completely ciliated. Eyes: two pairs, with lenses. Posterior adhesive organ situated ventrally with its posterior margin about 10 μ in front of the posterior extremity of the body, which is itself glandular. Hooks: 14, all of the same shape (Fig. 6) and 14 ± 0·5 μ long, without ogives; 12 hooks arranged equidistantly around the lateral and posterior margins of the disc, and 2 situated medianly and slightly anterior to the centre of the disc.

Entobdella soleae (Fig. 2)

Length 230–300 μ. Body with three bands of cilia as shown in diagram. Eyes: two pairs, with lenses. Posterior adhesive organ terminal. Hooks: 14 larval hooks, all of the same shape (Fig. 7) and 16 ± 0·5 μ long, and each with a pair of ogives each 11 ± 1 μ long. In addition, there are present the primordia of the three pairs of hooks of the adult, and of these the third or posterior-most pair is relatively well developed. The 14 larval hooks are arranged one pair immediately anterior to the first pair of adult hooks, one pair between the third pair of adult hooks, and the remaining five pairs arranged in two lateral rows as shown in Fig. 2.

Acanthocotyle lobianchi (Fig. 3)

Length 120–170 μ. Cilia absent. Eyes: absent. Posterior adhesive organ terminal. Hooks: 16, all of same shape (Fig. 8) and 26 ± 0·5 μ long, without ogives, with 14 arranged peripherally and equidistantly and two situated more centrally. An adhesive organ exactly similar to this larval structure is found immediately posterior to the large adhesive organ of the adult, thus it seems extremely probable that the larval adhesive organ survives unaltered throughout life.

Rajonchocotyle emarginata (Fig. 4)

Length 100–140 μ. Body with two bands of cilia as shown in diagram. Eyes: absent. Posterior adhesive disc terminal. Hooks: 10, all of the same shape (Fig. 9), 20 ± 0·5 μ long, each provided with a pair of ogives 12 ± 1·0 μ long, and arranged approximately equidistantly round the periphery of the adhesive organ. All of my preparations were slightly distorted, and I was unable to recognize the bilateral arrangement of hooks illustrated by Euzet (1955) for *Neoerpocotyle catenulata* (Hexabothriidae), but it is possible that such arrangement is present in *Rajonchocotyle*.

Diclidophoroidea (Fig. 5*a*, *b*)

Body, excepting adhesive organ, completely ciliated. Eyes: a single pair of laterally directed pigment cups without permanent lenses but with conspicuous oil droplets that probably function as lenses; paired nature of eyes easily recognizable in some, e.g. *Plectanocotyle*, but the two eyes so closely adpressed as to appear as one in others (e.g. *Pseudaxine*); eyes absent in *Diclidophora*. Body terminating posteriorly in a ciliated cone, the apex of which is glandular and from which a drop of viscous material in the course of being secreted may frequently be observed. This ciliated cone is probably a deciduous organ, since it is not recognizable after the shedding of the ciliated epidermis. Posterior adhesive organ ventral and bears a pair of lateral 'wings' on which are borne some of the larval hooks. Hooks: 12, without ogives, arranged in 6 pairs that exhibit serial differentiation into two or three kinds (Figs. 10–26). The hooks of the first four pairs (= lateral hooks) are invariably all alike in size and shape (Figs. 10–16), and are borne on the lateral wings of the adhesive organ; the hooks of the fifth pair (= postero-lateral hooks) are similar in shape to the lateral hooks, but may be larger (Figs. 17–19), and are borne more medianly than the lateral hooks; the hooks of the sixth pair (= posterior hooks, Figs. 20–26), are invariably larger than and of a different shape from the first five pairs, the particular shape and size varying with the species (Figs. 20–26).

Plectanocotyle gurnardi

Length 100–150 μ. Lateral and postero-lateral hooks all of same shape (Fig. 10) and all 13 ± 0·5 μ long. Posterior hooks (Fig. 20) 23 ± 0·5 μ long, with the shaft joined to the middle of the proximal border of the blade.

Anthocotyle merluccii

Length 160–200 μ. Lateral and postero-lateral hooks all of same shape (Fig. 11) and 16 ± 1·0 μ long. Posterior hooks (Fig. 21) 40 ± 2 μ long, with a long slender shaft attached to the blade at some little distance down the inner border of the blade.

Gastrocotyle trachuri

Length 160–200 μ. Postero-lateral hooks (21 ± 1 μ long, Fig. 17) longer than the lateral hooks (13 ± 0·5 μ, Fig. 12). Posterior hooks with shaft region expanded into a stout curved base (Fig. 22).

Pseudaxine trachuri

Length 160–200 μ. Lateral hooks (Fig. 13) indistinguishable from those of *Gastrocotyle*, postero-lateral hooks (19 ± 1 μ, Fig. 18) with shafts slightly shorter than those of *Gastrocotyle*. Posterior hooks (19 ± 1 μ, Fig. 23) similar in shape to those of *Gastrocotyle*, but smaller.

Microcotylid species from *Trachurus*

Length 100–140 μ. Lateral hooks and postero-lateral hooks all identical with each other (13 ± 0·5 μ, Fig. 14). Posterior hooks (32 ± 1 μ, Fig. 24) with long slender shaft attached at the junction of the proximal border of the blade to the inner border of the blade.

Microcotyle labracis

Length 200–240 μ. Lateral and postero-lateral hooks all identical with each other (18 ± 1 μ, Fig. 15). Posterior hooks similar in shape to those of previous microcotylid species, but larger (51 ± 1 μ, Fig. 25). In the newly hatched larva of *M. labracis* there are present the primordia of one of the pairs of hooks of the adult (Fig. 27).

Diclidophora merlangi

Length 210–250 μ. Postero-lateral hooks (27 ± 2 μ, Fig. 19) similar in shape to the lateral hooks (21 ± 2 μ, Fig. 16), but larger. Posterior hooks (31 ± 2 μ, Fig. 26) with outer margin of the proximal region of the blade sloping to meet the shaft.

COMPARISONS WITH OTHER MONOGENEAN LARVAE

Diplectanum aequans

The larva of *Diplectanum aequans*, in possessing 2 pairs of eyes and having 14 larval hooks, resembles the following larval gyrodactyloideans: *Dactylogyrus vastator*, described by Kulwieć (1929); *D. anchoratus*, described by Kulwieć (1927); *Neodactylogyrus macracanthus*, described by Wilde (1936); and *Acolpenteron ureteroecetes*, described by Fischthal & Allison (1942). *Diplectanum* differs from another gyrodactyloidean *Ancyrocephalus vistulensis*, described by Siwak (1932), only in that the latter is reported as having only 12 hooks, although Siwak's illustration of the larva appears to show 13 hooks. *Ancyrocephalus* was illustrated as having a posterior ciliated cone such as is present in *Diplectanum* and also in *Neodactylogyrus macracanthus*.

Entobdella soleae

The larva of *Entobdella soleae* bears a very close resemblance to the larva of *Benedenia melleni*, described by Jahn & Kuhn (1932), and differs from it only in the presence in the larval *Benedenia* of the anterior suckers which characterize the adult. The larvae of *Entobdella* and *Benedenia*, both capsaloideans, resemble the previously discussed gyrodactyloidean larvae in having 2 pairs of eyes and 14 larval hooks, but in the capsaloideans primordia of the 3 pairs of adult hooks are also present.

Acanthocotyle lobianchi

The larva of *Acanthocotyle lobianchi*, as described in the present study, resembles the embryo of *A. pugetensis*, as described by Bonham & Guberlet (1938), and thus differs slightly in the arrangement of its 16 hooks from the embryonic *A. pacifica*, which was also described by Bonham & Guberlet. Of other young forms of Monogenea, the embryos and larvae of *Acanthocotyle* with their 16 hooks and absence of eyes and cilia resemble most the embryo of *Gyrodactylus elegans*, described by Katheriner (1904). The significance of this similarity may, however, be slight when the clearly abnormal polyembryonic development of *Gyrodactylus* is considered.

Rajonchocotyle emarginata

The larva of *Rajonchocotyle emarginata*, with its absence of eyes and its 10 hooks, resembles very closely the larva of *Neoerpocotyle catenulata* described by Euzet (1955) and belonging to the same family, the Hexabothriidae.

Diclidophoroidea

Among the Diclidophoroidea the only previously known larval discocotylid is *Diplozoon paradoxum*, described by Zeller (1872*a*). This species differs from *Plectanocotyle gurnardi* and *Anthocotyle merluccii* in that the newly hatched young does not have a larval adhesive organ, the clamps of the adult being already present. One pair of hooks is present, and these persist in the adult, but insufficient is known of their development to permit comparisons with the larval hooks of other species. In common with other diclidophoroideans, the newly hatched *Diplozoon* has 2 eyes which disappear in the adult.

The larvae of *Microcotyle labracis* and of the 'unidentified microcotylid species' from *Trachurus trachurus* resemble the larvae of *Microcotyle chrysophrii* and *Axine bellones*, described by Euzet (1955), in that they are diclidophoroideans having the lateral and postero-lateral hooks all similar to each other, and in that the shapes of the posterior hooks in all four species are all very similar. In all these microcotylideans except the one from *Trachurus*, a seventh pair of hooks, which appears to be primordia of adult hooks, is present. Such hooks are also present in the larva of *Microcotyle spinicirrus*, described by Remley (1942), and very probably in *Diplasiocotyle johnstoni* also, but Sandars's (1944) description of the latter species was insufficiently detailed to be certain about this point. *Microcotyle spinicirrus* differs from all the other known microcotylid larvae in that it has *six* pairs of lateral and postero-lateral hooks in addition to its posterior hooks and its primordial adult hooks.

The larvae of *Gastrocotyle trachuri* and *Pseudaxine trachuri* resemble each other very closely indeed, and in fact are only distinguishable from each other in the slight differences in size of their hooks (see pp. 251–2). Both species differ from the other members of the Microcotylidae, in which they are at present classified, in the differences between lateral and postero-lateral hooks, in the very different shapes of the posterior hooks, and in the absence of primordia of adult hooks.

A comparison of the larva of *Diclidophora merlangi* with those of *D. denticulata*, described by Frankland (1955), and *D. luscae*, described by Gallien (1934), reveals no differences excepting in the relative sizes of the lateral and postero-lateral hooks. Gallien (1934), without giving a separate description, stated that the structure of the adhesive organ of the larva of *D. pollachii* was identical with that of *D. luscae*.

The above comparisons have included reference to all the known larval monogeneans excepting the two diclidophoroideans *Kuhnia scombri*, described by Gallien & Le Calvez (1947), and *Hexostoma thynni*, described by Euzet (1955), and the Polystomatidae *Polystoma integerrimum*, described by Zeller (1872*b*), Halkin (1901), and Gallien (1935); *Sphyranura oligorchis*, described by Alvey (1936); *Polystoma nearcticum* and *Polystomoidea oris*, both described by Paul (1938); and *Diplorchis scaphiopodis*, described by Rodgers (1941).

Kuhnia scombri differs from other larval diclidophoroideans in that the hooks are all similar, there being no differentiation into lateral, postero-lateral and posterior hook types, and in that no eyes are present. *Hexostoma thynni* resembles the discocotylideans *Plectanocotyle* and *Anthocotyle* in its equipment of hooks, but differs from all other diclidophoroideans in that it has 2 pairs of eyes and a curious asymmetrical longitudinal band of pigment. The five larval Polystomatidae all resemble each other in having 16 larval hooks with one or 2 pairs of primordial adult hooks. Four of the species have two pairs of eyes, but in the fifth species *Sphyanura osleri*, which hatches in an advanced state of development, eyes (and cilia) are absent. The polystomatid larvae are thus quite similar to the larval gyrodactyloideans and capsaloideans from which they differ in having 16 instead of 14 larval hooks.

DISCUSSION

Monogenean larvae were in the past variously referred to as 'miracidia' or merely as 'ciliated larvae', until Gallien (1934) proposed the term 'gyrodactyloid larva' for the larvae of *Diclidophora* and *Polystoma*, on account of their resemblance to *Gyrodactylus*. However, *Gyrodactylus* has a very abnormal embryonic development, and the adult does not have the cilia or eye-spots characteristic of most monogenean larvae. Moreover, the use of a term which is also used to refer to the members of one particular superfamily of the Monogenea could cause confusion when it is desired to refer to the larvae of species belonging to other superfamilies. Thus in place of Gallien's 'gyrodactyloid larva' as a name for monogenean larva, I propose the substitution of 'oncomiracidium' (Greek, *onkos*, hook; *meirakidion*, youth), which suggests affinity with the familiar digenean miracidium and reflects the presence of the characteristic monogenean hooks.

The contribution that studies of larvae may make to the study of the phylogeny of the Monogenea will now be considered. If the 33 oncomiracidia now known (*Diplozoon* and *Sphyranura* are omitted because of their very advanced development on hatching) are arranged according to their complements of eyes and hooks, the following groups readily emerge.

1. Gyrodactyloideans (except *Gyrodactylus*) and capsaloideans, both with 14 larval hooks and 2 pairs of eyes with lenses (9 spp.).
2. Polystomatidae with 16 larval hooks and 2 pairs of eyes with lenses (4 spp.).
3. Diclidophoroideans with 6 pairs of larval hooks exhibiting serial differentiation, and with 0, 1, or 2 pairs of eyes without permanent lenses (16 spp.).
4. Hexabothriidae with 10 larval hooks and without eyes (2 spp.).
5. *Acanthocotyle* and *Gyrodactylus*, both with 16 hooks and without eyes. On account of the abnormal development of *Gyrodactylus* and the difference in hosts (one on elasmobranchs, the other on teleosts), the resemblance of these species may be no more than superficial (2 spp.).

This grouping differs from the present classification of adults (Sproston, 1946), mainly in the divorce of the family Hexabothriidae from its place alongside the Polystomatidae in the superfamily Polystomatoidea, and in the inference of a closer affinity than is now recognized between the Polystomatidae on the one hand, and the Gyrodactyloidea and Capsaloidea on the other. A consideration of the affinities between the respective hosts yields strong support for the re-arrangement since the Hexabothriidae parasitize Chondrichthyes, the Polystomatidae parasitize Amphibia and Reptilia, and both the Gyrodactyloidea and Capsaloidea parasitize Teleostii.

It becomes pertinent therefore to examine the criteria used at present in the classification (Sproston, 1946) of the Monogenea. The main diagnostic features separating the two suborders Monopisthocotylea and Polyopisthocotylea (including the Hexabothriidae, Polystomatidae and Diclidophoroidea) are the relationship of the adhesive organs of the adult to those of the larva, and the presence or absence of a genito-intestinal canal.

The use of the first of these characters is of extremely doubtful validity. The Polyopisthocotylea were defined as being Monogenea in which (among other characters) the functional haptor (=adhesive organs) of the adult is developed immediately anterior to the larval haptor. So far as I can discover, this conception can only be based upon what appears to be a misinterpretation of some rather confused observations by Remley on the development of *Microcotyle spinicirrus*. Remley (1942, p. 151) stated that the larval haptor of *M. spinicirrus* bore 6 pairs of hooklets (=lateral and postero-lateral hooks in my descriptions) along the lateral and posterior border, and that 2 pairs of large anchor hooks (=posterior hooks and primordial adult hooks in my descriptions) were medianly located near the posterior end. Later on p. 151, and on p. 152, Remley used the term 'larval haptor' in a different sense—to include only that part bearing the 'anchor hooks', and when he stated that by the 20 to 30-clamp stage the haptor was lost, he was referring only to the anchor-bearing part. There are no grounds then for supposing that the adhesive organs of the adult do not develop around and replace the larval hooks as they have been described to do so in *Diclidophora denticulata* by Frankland (1955) and in several polystomatids by various authors.

The use of the second character appears to be more valid: a genito-intestinal canal is invariably present in the Polyopisthocotylea, but has been reported as being present also in the Protogyrodactylidae and in some turbellarians, and so it is possible that its occurrence throughout the Polyopisthocotylea is due to convergence. However, the genito-intestinal canal in the Polyopisthocotylea may be associated with another feature that may be shown eventually to be exclusive to the group: I have shown that in eight representative species the gut epithelium consists of scattered 'pigment cells' associated with a blood-feeding habit (Llewellyn, 1954), and it is possible that there may be a connexion between an obligatory blood-feeding habit and the presence of a genito-intestinal canal. It is known (see Caullery, 1952) that there is a close correlation between strict haematophagy and the presence of intestinal symbionts, and that such blood-feeding animals are obliged to have some means of transferring the symbionts to succeeding generations. I have observed cells laden with bacteria (=mycetocytes?) in the intestine of adult specimens of *Axine bellones*, in the egg capsules of *Polystoma integerrimum*, and in the egg capsules of most of the diclidophoroideans that I have described in this paper. The genito-intestinal canal *could* be the pathway whereby such mycetocytes may be transferred from the gut to the egg capsules. If this hypothesis be shown eventually to be correct, the inference would be of a common origin for those monogeneans having a blood-feeding habit, gut pigment cells, and a genito-intestinal canal. This in turn would mean that the similarity between the larvae of the Polystomatidae and those of the

Capsaloidea is superficial unless it be due to the present-day Polystomatidae being in fact those Polyopisthocotylea which have diverged least from an ancestral stock that is common also to the Capsaloidea.

Within the present suborder Monopisthocotylea, the inference from larval studies is that there is a closer affinity between the Gyrodactyloidea (excepting the Gyrodactylidae) and the Capsaloidea than is suggested by the present classification, and this is supported by the widespread occurrence of 14 marginal hooks (=persistent larval hooks?) in many adults of both the Gyrodactyloidea and the Capsaloidea.

With regard to the Diclidophoroidea, while an insufficient number of species is known yet to permit a classification of the group on larval characters, it is possible to make the following observations: (1) In *Kuhnia scombri* (Mazocraeidae) the presence of undifferentiated hooks appears to be the survival of a primitive character, and Sproston (1945) has already regarded the clamp structure of the adult *Kuhnia* to be the basic type from which other diclidophoroidean clamps have evolved—a view which I have opposed (Llewellyn, 1957). (2) The larvae of *Gastrocotyle trachuri* and *Pseudaxine trachuri* (at present classified in the Microcotylidae) appear to have less in common with microcotylid larvae than the microcotylids have in common with the discocotylids, diclidophorids, and hexastomatids. This would support Price's (1943) proposal, on the grounds of the clamp structure of the adults, of a new family Gastrocotylidae.

It is again a pleasure to record my thanks to the Director and Staff of the Plymouth Laboratory for providing excellent working facilities. I am especially grateful to Mr G. R. Forster for supplies of *Morone labrax*, to Mr A. D. Mattacola for assistance with fish matters, and to Mr J. E. Green for help in collecting parasites.

SUMMARY

Techniques for the rearing of monogenean larvae are described.

The new term 'oncomiracidium' is proposed for the larvae of monogenetic trematodes.

Eleven new oncomiracidia of fish parasites are described, bringing the total now known to thirty-five.

These oncomiracidia are tentatively classified, and the classification is compared with the existing classification of adult monogeneans.

REFERENCES

Alvey, C. H., 1936. The morphology and development of the monogenetic trematode, *Sphyranura oligorchis* Alvey, 1933, and the description of *Sphyranura polyorchis* n.sp. *Parasitology*, Vol. 28, pp. 229–53.

Baer, J. G., 1951. *Ecology of Animal Parasites.* 224 pp. Urbana: University of Illinois Press.

Bonham, K. & Guberlet, J. E., 1938. Ectoparasitic trematodes of Puget Sound fishes. *Acanthocotyle. Amer. Midl. Nat.*, Vol. 20, pp. 590–602.

Caullery, M., 1952. *Parasitism and Symbiosis.* 340 pp. London: Sidgwick and Jackson.

Dawes, B., 1946. *The Trematoda.* Cambridge University Press.

Euzet, L., 1955. Larves gyrodactyloïdes nageantes de quelques trématodes monogénétiques de poissons marins. *Bull. Soc. neuchâtel. Sci. nat.*, T. 78, pp. 71–9.

Fischthal, J. H. & Allison, L. N., 1942. *Acolpenteron catastomi* n.sp. (Gyrodactyloidea: Calceostomatidae), a monogenetic trematode from the ureters of suckers, with observations on its life history and that of *A. ureteroecetes. Trans. Amer. micr. Soc.*, Vol. 61, pp. 53–6.

Frankland, H. M. T., 1955. The life history and bionomics of *Diclidophora denticulata* (Trematoda: Monogenea). *Parasitology*, Vol. 45, pp. 313–51.

Gallien, L., 1934. Sur la larve de *Dactylocotyle luscae* v. Ben. and Hesse, trématode monogénétique marin. *Bull. Soc. zool. Fr.*, T. 59, pp. 68–73.

—— 1935. Recherches expérimentale sur le dimorphisme évolutif et la biologie de *Polystomum integerrimum* Froel. *Trav. Sta. zool. Wimereux*, T. 12, pp. 1–182.

Gallien, L. & Le Calvez, J., 1947. Description de la larve d'*Octobothrium scombri* van Beneden & Hesse, trématode monogénétique marin. *Bull. Soc. zool. Fr.*, T. 72, pp. 76–8.

Halkin, H., 1901. Recherches sur la maturation, la fécondation et le développement du *Polystomum integerrimum. Arch. Biol. Paris*, T. 18, pp. 291–363.

Jahn, T. L. & Kuhn, L. R., 1932. The life-history of *Epibdella melleni* MacCallum, 1927, a monogenetic trematode parasitic on marine fishes. *Biol. Bull., Woods Hole*, Vol. 62, pp. 89–111.

Katheriner, L., 1904. Über die Entwicklung von *Gyrodactylus elegans* v. Ndm. (Abstract). *Zool. Jb.*, Bd. 7, suppl., pp. 519–51.

Kulwieć, Z., 1927. Untersuchung an Arten des Genus *Dactylogyrus* Diesing. *Bull. int. Acad. Cracovie (Acad. pol. Sci.) sci. nat.*, Ser. B (1–2), pp. 113–44.

—— 1929. Observations sur le développement de *Dactylogyrus vastator* Nyb. *Arch. Hydrobiol. Ichthiol.*, T. 4, pp. 277–86.

Llewellyn, J., 1954. Observations on the food and gut pigment of the Polyopisthocotylea (Trematoda: Monogenea). *Parasitology*, Vol. 44, pp. 428–37.

—— 1956. The host-specificity, micro-ecology, adhesive attitudes, and comparative morphology of some trematode gill parasites. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 113–27.

—— 1957. The mechanism of the attachment of *Kuhnia scombri* (Kuhn, 1829) (Trematoda: Monogenea) to the gills of its host *Scomber scombrus* L., including a note on the taxonomy of the parasite. *Parasitology*, Vol. 47 (in the Press).

Paul, A. A., 1938. Life-history studies of North American fresh-water polystomes. *J. Parasit.*, Vol. 24, pp. 489–510.

Price, E. W., 1943. North American monogenetic trematodes: VI. The family Diclidophoridae (Diclidophoroidea). *J. Wash. Acad. Sci.*, Vol. 33, pp. 44–54.

Remley, L. W., 1942. Morphology and life-history studies of *Microcotyle spinicirrus* MacCallum, 1918, a monogenetic trematode parasitic on the gills of *Aplodinotus grunniens. Trans. Amer. micr. Soc.*, Vol. 61, pp. 141–55.

Rodgers, L. O., 1941. *Diplorchis scaphiopi*, a new polystomatid monogenean fluke from the Spadefoot Toad. *J. Parasit.*, Vol. 27, pp. 153–7.

Rowan, W. B., 1956. The mode of hatching of the egg of *Fasciola hepatica. Experimental Parasitology*, Vol. 5, pp. 118–37.

Sandars, D. F., 1944. A contribution to the knowledge of the Microcotylidae of Western Australia. *Trans. roy. Soc. S. Aust.*, Vol. 68, pp. 67–81.

SIWAK, J., 1932. *Ancyrocephalus vistulensis* n.sp., un nouveau trématode, parasite du Silure (*Silurus glanis* L.). *Bull. int. Acad. Cracovie* (*Acad. pol. Sci.*), *sci. nat.*, Ser. B II (7–10), pp. 669–79.

SPROSTON, N. G., 1945. A note on the comparative anatomy of the clamps in the superfamily Diclidophoroidea (Trematoda: Monogenea). *Parasitology*, Vol. 36, pp. 191–4.

—— 1946. A synopsis of the monogenetic trematodes. *Trans. zool. Soc. Lond.*, Vol. 25, pp. 185–600.

STUNKARD, H. W., 1937. The physiology, life cycles and phylogeny of the parasitic flatworms. *Amer. Mus. Novit.*, no. 908, 27 pp.

WILDE, J., 1936. *Dactylogyrus macracanthus* Wegener als Krankheit-serreger auf den Kiemen der Schleie (*Tinca tinca* L.). *Z. Parasit.*, Bd. 9, pp. 203–36.

ZELLER, E., 1872*a*. Untersuchungen über die Entwicklung des *Diplozoon paradoxum*. *Z. wiss. Zool.*, Bd. 22, pp. 168–80.

—— 1872*b*. Untersuchungen über die Entwicklung und Bau des *Polystomum integerrimum* Rud. *Z. wiss. Zool.*, Bd. 22, pp. 1–28.

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LUMINESCENCE IN POLYNOIDS

II. DIFFERENT MODES OF RESPONSE IN THE ELYTRA

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(Text-fig. 1)

Earlier studies (Nicol, 1953, 1954) showed that two kinds of luminescent responses are produced by electrical stimulation of isolated polynoid scales, viz. brief flashes and a long-lasting glow, the latter frequently being brighter. The brief flashes were regarded as the normal response of the photocytes to nervous excitation, whereas the long glow might be the result of direct excitation of the photocytes. A prolonged glow can usually be evoked by strong shocks (well above threshold). It is sometimes succeeded by rhythmic flashing (Nicol, 1954, fig. 1 B); alternatively, a series of rhythmic flashes may be succeeded by a prolonged glow (Nicol, 1954, fig. 3 A).

In the present paper I shall consider the two kinds of responses in more detail.

MATERIAL AND METHODS

The species studied were *Polynoë scolopendrina*, *Lagisca extenuata* and *Gattyana cirrosa*; most observations were made on *Polynoë*. Two kinds of preparations were used. These were isolated elytra, and short sections of worm consisting of a few segments and an elytrum. The material was prepared from worms narcotized with iso-osmotic $MgCl_2$. To induce luminescence electrical stimulation was employed, either condenser discharges or square waves, delivered from an electronic apparatus. The isolated elytrum was placed in a moist chamber over a pair of silver electrodes. To stimulate pieces of the body, silver electrodes were placed on the nerve cord. Luminescent responses were detected with photomultiplier, d.c. amplifier and cathode-ray oscilloscope. Records were made on moving paper. Temperatures were $18^\circ \pm 1^\circ$ C.

OBSERVATIONS

STIMULATION THROUGH THE NERVE CORD

The following observations were made on short sections of worm containing an elytrum, and stimulated via the ventral nerve cord.

Polynoë. A single shock sometimes produces one flash, or causes repetitive flashing; or several shocks may be required to elicit the first recordable response. Fast repetitive flashing occurs at frequencies up to 10/sec.

Consecutive flashes show facilitatory increment. Flashing follows pulse-rate up to about 20/sec, above which there is no longer a flash to each shock. Following several flashes at short intervals, the final flash may pass over into a glow response, having a lengthened decay period (Fig. 1 A). A normal quick flash has a duration of some 120 msec, and rise time of *ca.* 25 msec; decay time is about 100 msec. The decay time of the glow response is variable and prolonged, from 0·25 to 3 sec. When a flash gives way to a prolonged glow, there may be an initial period of fast decay, followed by a secondary rise and slow decay (Fig. 1 A). I would emphasize that the prolonged glow may appear long after the stimulatory shock has been delivered (0·3–0·6 sec).

Gattyana. A single shock elicits either a flash or repetitive flashing. Flashing follows stimulation up to pulse-rates of about 24/sec (Fig. 1 B), above which the scale does not respond to each shock. During a high-frequency burst, repetitive flashing tends to give way to a steady glow, but prolonged glow responses are not otherwise apparent in these records. Consecutive flashes show facilitation. Flashes vary in duration from 54 to 145 msec; rise time is 8–25 msec. In a series of flashes, later ones, of no greater amplitude than the first, may have greater duration and rise time.

Stimulation of the nerve cord in narcotized pieces

Short sections of worm (*Polynoë*) containing nerve cord and one elytrum were narcotized for 4 h or longer in 0·2 % novocaine, and then stimulated via the nerve cord. Weak luminescent responses could only be obtained with very strong shocks (5 × threshold of unanaesthetized preparations). These were glow responses, rather than quick flashes. I assume there was spread of current at the very high voltages to the underlying elytrum, which was excited directly.

ELECTRICAL STIMULATION OF ISOLATED ELYTRA (NARCOSIS-FREE)

Polynoë. In an isolated elytrum a single shock produces either one flash, or a series of flashes, variable in number. Temporal characteristics are similar to those recorded in a previous section (latency, 13–21 msec; duration, *ca.* 100 msec; rise time, 26–30 msec) (Nicol, 1953). Consecutive flashes show facilitation (Nicol, 1954). With bursts of shocks, the scale gives off a train of flashes. At high frequencies (> 20/sec), the scale either follows the rate of stimulation, or flashes to every 2nd or 3rd pulse. Light pulsations at rates up to 64/sec have been observed. The responses at high rates are of low intensity, and when stimulation ceases the scale continues to flash at some lower rate (*ca.* 10/sec) and higher intensity (Fig. 1 C). After a bout of repetitive flashing, induced by one or multiple shocks, there is often a prolonged glow with long decay period (up to 8 sec). Fast repetitive flashes are sometimes superposed on background glow. Strong shocks (2 × threshold) induce rapid repetitive flashing accompanied by a prolonged glow response. Decay of the latter occupies about 2 sec.

Gattyana. Isolated scales of *Gattyana* tend to give the prolonged glow response, even when stimulated at threshold. A single shock evokes responses of the following kinds: (1) initial quick flash followed by a prolonged glow; (2) rapid repetitive discharge passing over into a prolonged glow; (3) prolonged glow, with longer rise and decay time than a quick flash (Fig. 1 D); (4) prolonged glow passing into repetitive flashing (Nicol, 1953, fig. 9). In some records the initial repetitive flashes return to base-line (zero intensity), whereas later responses become superposed on a rising background. Latency of the prolonged glow response varies from *ca.* 25 to 100 msec; rise time is 0·25 sec; duration is 4–8 sec.

Lagisca. A shock near threshold usually evokes a flash or a bout of repetitive flashing. When rapid, the latter may end in a prolonged glow response. The maximal rate of rhythmic flashing to a single shock is *ca.* 10/sec. Normal flashes follow repetitive stimulation up to frequencies of about 12/sec. At higher frequencies the scale responds to every 2nd, 3rd, or other stimulus with normal flashes; or closely follows the stimulus-rate with weak pulsations, which give way to bright normal flashes when stimulation ceases (Nicol, 1954, fig. 4 C, D). Consecutive flashes show facilitation (Fig. 1 E). Flash duration varies from 100 to 200 msec; rise time is 17–50 msec. In a series of flashes later ones tend to be more prolonged. Occasionally, a single shock evokes a glow response, with slow rise (*ca.* 0·3 sec) and long decay (>2 sec) (Nicol, 1954, fig. 1 A). Following rapid flashing, there may be a glow response (Nicol, 1954, fig. 3 A).

STIMULATION OF NARCOTIZED ELYTRA

Isolated elytra were placed in solutions of certain anaesthetics, and their responses to electrical stimulation were tested later. Narcotics used were: cocaine, 0·2%; novocaine, 0·2–0·5%; chloretone, 0·05–0·2%; M.S.222 (Sandoz), 0·025–0·1%; all in sea water. Also employed were iso-osmotic solutions of $MgCl_2$ plus sea water (ratios 1:3 to 1:1).

Evidence, presented elsewhere, has shown that continued rhythmic flashing, after stimulation has ceased, is dependent upon an elytral ganglion. Multiple flashes to a single shock, therefore, have been used as an index of continued nervous functioning. Multiple flashing (two or more flashes) to a single shock was observed in scales immersed for 97 min in 0·1% chloretone, and 54 min in 0·2% novocaine. Immersion times were considered insufficient, and the following observations are based on elytra immersed in narcotic solutions for 4 h or more.

Novocaine 0·2%

Polynoë. Responses of several kinds to single pulses were recorded as follows: (1) quick flash with latency *ca.* 10 msec, rise time 20 msec, duration *ca.* 0·5 sec; (2) initial quick flash followed by a long glow: rise time 20–80 msec, duration up to 10 sec (Fig. 1 F); (3) prolonged glow response having a slow rise and long decay period: rise time 0·3 sec, duration 7–8 sec (Fig. 1 G). These various kinds of responses are really arbitrary, and I believe there is every gradation in narcotized *Polynoë* scales between a brief flash lasting a half second to a prolonged glow lasting many seconds. Rise time is equally variable. Some records show an initial quick flash in which the decay period is interrupted by a secondary slow rise, succeeded by prolonged plateau and slow decay (Fig. 1 H). With repetitive stimulation discrete flashes have been recorded up to rates of 18/sec. In some records there is little or no facilitatory increment in consecutive responses; in other records facilitation is well marked in the first few responses (Fig. 1 J).

Gattyana. Only glow responses were observed. These have a latency of *ca.* 27 msec, and duration of 5–9 sec. Rise time is sometimes long, up to 1 sec, but many responses begin with an initial quick flash (rise time some 30 msec) which passes over into a prolonged glow. Occasionally, there is a secondary slow rise after the initial quick flash has started to decay. Facilitation to consecutive pulses is sometimes marked.

Lagisca. Single shocks produced a flash, repetitive flashing, or a prolonged glow. Repetitive flashing was sometimes succeeded by a prolonged glow response. The latency of the glow response was rather long, 0·5–1 sec; rise to maximum was 0·4 sec; total response duration was 3–4 sec. Because of the repetitive flashing observed, it appears that *Lagisca* elytra are not always narcotized by novocaine at 0·2% level.

Novocaine 0·3%

Lagisca. Stimulation produced glow response. Latency *ca.* 20 msec; duration some 10 sec; rise time 0·7 sec. The first two of a series of responses show facilitatory increment.

Novocaine 0·5%

Lagisca. Nil response.

Cocaine 0·2%

Polynoë. Following treatment with cocaine, the following kinds of responses were observed.

(1) Quick initial flash followed by a long glow.

(2) Quick initial flash, with decay period interrupted by a secondary rise.

(3) A quick flash showing rapid return to base-line (Fig. 1 K). Latencies are 13–14 msec; rise time is *ca.* 27 msec. Minimal flash duration is about 0·25–0·3 sec; the more prolonged glows last up to 8 sec. With repeated stimulation, the response becomes more prolonged. Facilitation is shown in consecutive flashes (Fig. 1 K).

Chloretone 0·05 %

Polynoë. Responses obtained after chloretone show rapid rise (*ca.* 30 msec), and slow decay (2–4 sec). Latency is *ca.* 15 msec. In some records the decay curve is regular; in others an initial decay is followed by a slow secondary rise and long period of secondary decay. There is facilitation to consecutive flashes (Fig. 1 L–N).

*M.S.*222, 0·025 %

Polynoë. Responses were a prolonged glow, lasting up to 3 sec, with slow rise time (0·25–0·3 sec). The glow response usually was preceded or accompanied by a few weak oscillations, almost imperceptible in the records. Latency varied in different records from 0·025 to 0·4 sec. The small pulses accompanying the prolonged glow would indicate some residual nervous excitation, and this concentration of M.S. 222 can be considered near threshold.

*M.S.*222, 0·05 %

Polynoë. Pulses produce a long glow (duration up to 7 sec). Rise time varies from 0·25 to 1 sec. Facilitation is shown in consecutive responses.

*M.S.*222, 0·1 %

Polynoë. Nil response to electrical stimulation.

$MgCl_2$: *sea water* 1:3

Polynoë. Single shocks usually elicited multiple flashing, with responses similar to those of non-narcotized scales. Occasional scales gave the prolonged glow response. Consecutive flashes showed facilitation.

$MgCl_2$: *sea water* 1:2

Polynoë. Pulses evoked quick flashes passing into a prolonged glow, or repetitive flashing sometimes ending in a glow response. Facilitation was evident.

$MgCl_2$: *sea water* 2:3

Polynoë. Single flashes tended to be prolonged. Stimulation sometimes evoked a glow response, on which was superposed repetitive flashing.

$MgCl_2$: *sea water* 1:1

Polynoë. Nil response.

COMMENT

Treatment with each of three narcotics, viz. novocaine, chloretone, and M.S. 222, abolishes repetitive flashing to a single shock. This result is taken to indicate that anaesthesia stops nervous excitation and transmission. The

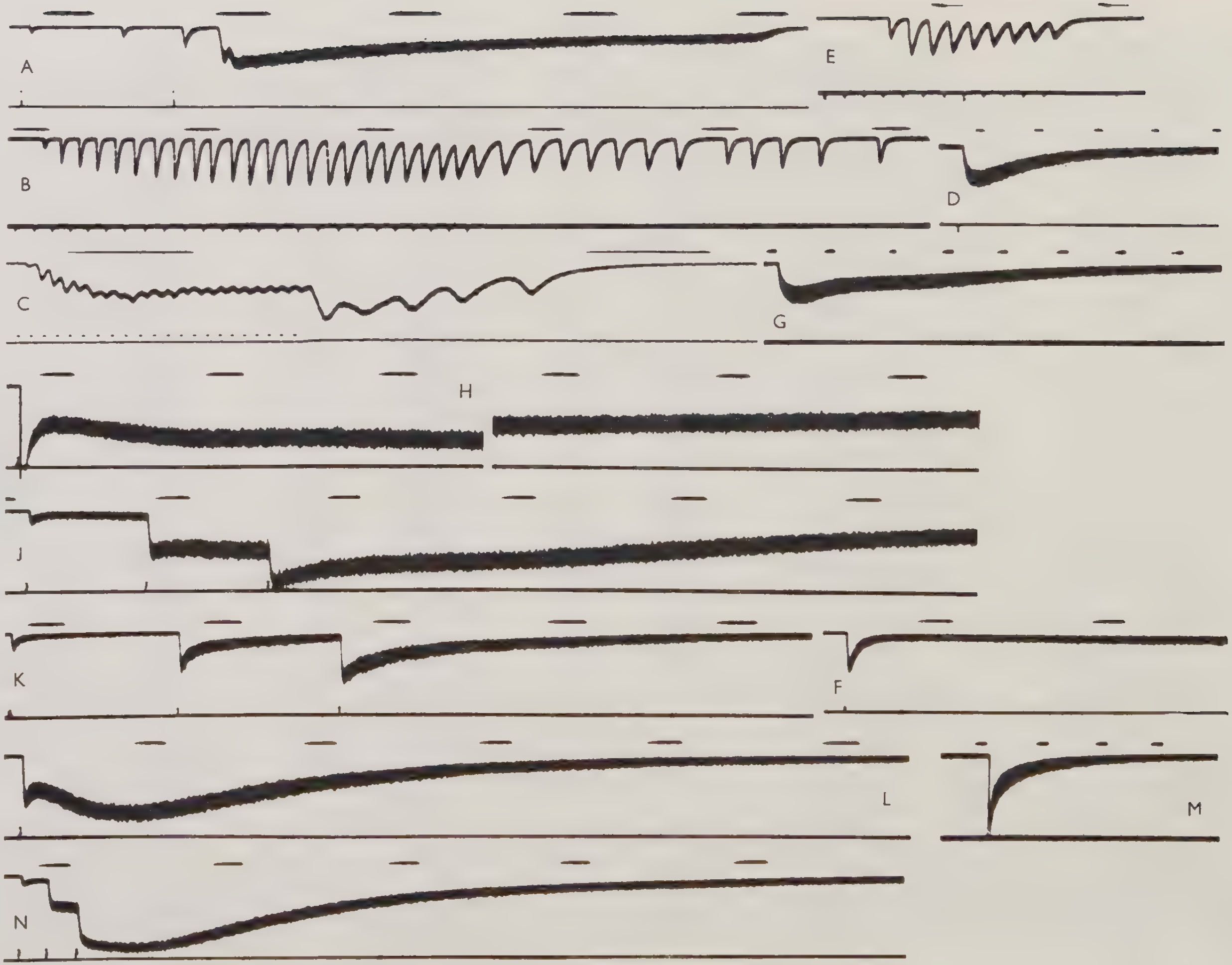

Fig. 1

luminescent responses which are still obtained by electrical stimulation after anaesthesia presumably arise from direct excitation of the photocytes.

Rhythmicity and nervous conduction are admittedly two different phenomena, although the same neurones are concerned with both. A possibility remains, therefore, that nervous conduction is still occurring, even though rhythmic discharge has disappeared. In view of the concentrations of narcotics employed, and the duration of their action, this contingency seems improbable.

Two kinds of responses have been arbitrarily selected, viz. the quick flash (duration *ca.* 100 msec), and the prolonged glow (duration 0·25 sec or longer). The former only appears after nervous stimulation (i.e. in unanaesthetized scales); the latter, following nervous stimulation or direct excitation (i.e. in unanaesthetized and in anaesthetized scales). It has not been possible to produce the quick flash by direct excitation of anaesthetized scales.

There are several reasons for believing that the glow response can be induced by nervous excitation, even under conditions when the isolated scale is stimulated. First, it is produced by stimulation of the nerve cord, when there is no current escape to the scale. Secondly, it succeeds rapid flashing some considerable time after stimulation has ceased. When evoked by indirect excitation, via the nervous system, the prolonged glow is associated with fast repetitive flashing. It seems that the conditions obtaining during fast repetitive flashing create a state favouring prolonged luminescence.

It is not known where the excitation takes place that produces the long glow response. Minimal latent periods of quick flash and glow response are about the same, and provide no help in resolving the question. Conduction time would be very brief in the short distances involved. Since quick flashes

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Legend to Fig. 1

Fig. 1. Oscillograph records

A. Luminescent responses of *Polynoë scolopendrina.* The preparation consisted of a piece of the body containing 3 segments plus 1 elytrum. Electrical stimuli were applied to the nerve cord. Downward deflexions of middle trace are light flashes. Time signal above, 1 sec. Electrical stimuli on lower line.

B. *Gattyana cirrosa.* Repetitive stimulation of the nerve cord. Recording from one elytrum.

C. *Polynoë scolopendrina.* Responses of an isolated elytrum to repetitive electrical stimulation (burst at 42/sec).

D. *Gattyana cirrosa.* Prolonged glow response of an isolated elytrum.

E. *Lagisca extenuata.* Flashing of an isolated scale to repetitive stimulation.

F, G. *Polynoë scolopendrina.* Glow responses of a scale in 0·2 % novocaine, 4 h 20 min.

H. *P. scolopendrina.* Glow response of elytrum in 0·2 % novocaine, 5 h 47 min. Five-second interval between the first and second halves of this record.

J. *P. scolopendrina.* Three consecutive glow responses of an elytrum in 0·2 % novocaine, 5 h 11 min.

K. *P. scolopendrina.* Consecutive responses of a scale in 0·2 % cocaine, 4½ h.

L, M, N. *P. scolopendrina.* Glow responses of a scale in 0·05 % chloretone, 4 h 25 min.

are sometimes superposed on the prolonged glow response, it follows that the transmission of seriated impulses and repeated excitation of the photocytes are not impeded during the lengthy course of the glow response.

The glow response often has a rapid increment, with rise time equal to the quick flash. Decay time greatly exceeds that of the flash, from 0·25 to 10 sec. A secondary rise in intensity, seen in many records, must be of more than fortuitous significance.

After a bright glow response the scale fails to respond, or gives only feeble responses, to further stimuli. The prolonged glow consumes much or all of some substance essential for the luminescent reaction.

Weak stimulation of unanaesthetized scales evokes quick flashes; strong electrical stimulation induces the glow response. Weak shocks excite the nervous system; stronger shocks excite the photocytes directly. These differences need not be one of threshold; they may be due to the geometrical relations of the excitable elements with reference to the cathode.

It has been observed frequently in narcotized scales that repetitive stimulation can engender facilitatory increment (staircase). This is seen most clearly at slow rates of stimulation, when the effect is not obscured by summation. In facilitation some improving effect of a response is carried over to a later response, appearing in a brighter flash. Since the nervous system is excluded, the effect must be ascribed to processes taking place in the photocyte. The facilitatory increment seen in flashes of non-narcotized scales may also depend upon processes occurring within the photocytes, although concomitant neuro-photocyte facilitation is not excluded.

During repetitive flashing later responses may become prolonged. This lengthening of decay period may be due to conditions similar to those responsible for the glow response.

Finally, it has been noted that normal flashing ceases to maintain correspondence with stimulation at rates above *ca.* 20/sec; weak pulsations are sometimes seen at much higher rates, when the isolated scale is stimulated. This problem remains unresolved. It is possible that this very fast pulsation, above that which seems permitted by the refractory period of the nerve, depends upon irregular rotation among a large population of nerve fibres.

I conclude that the prolonged glow response is physiologically possible, and is sometimes produced by fast repetitive excitation from the nervous system. The latter creates conditions favouring a massive luminescent discharge. The duration of a glow response can be greatly exceeded by a bout of protracted iterative flashing; the light, of course, is fluctuating in the latter condition.

Since facilitation or staircase is observed in anaesthetized scales, it appears to depend upon processes taking place within the photocytes.

SUMMARY

Elytra of polynoids show two kinds of luminescent responses, viz. a quick flash (duration *ca.* 100 msec) and prolonged glow (duration 0·25–10 sec). Quick flashes are produced by nervous excitation; prolonged glow responses accompany or follow fast repetitive flashing, or are elicited by strong electrical stimuli. Anaesthetized isolated elytra still give the prolonged glow response when electrically stimulated. Facilitation is also evident in consecutive glow responses. From these various observations it is concluded that the prolonged glow response can be produced by fast repetitive nervous excitation or by direct electrical excitation of the photocytes. The occurrence of facilitation in glow responses of anaesthetized elytra raises the problem whether the same phenomenon in consecutive normal flashes may be due to intracellular changes taking place within the photocytes.

REFERENCES

NICOL, J. A. C., 1953. Luminescence in polynoid worms. *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 65–84.

—— 1954. The nervous control of luminescent responses in polynoid worms. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 225–55.

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LUMINESCENCE IN POLYNOIDS

III. PROPAGATION OF EXCITATION THROUGH THE NERVE CORD

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Luminescent polynoids emit light from their elytra. These structures are arranged in two longitudinal rows covering most or all of the dorsal surface of the worm. Light emission is evoked by stimulation and appears as a single flash or series of flashes (Nicol, 1953, 1957).

When a polynoid is subjected to tactile stimulation, scales anterior and posterior to the stimulated region emit flashes. The response is a reflex, the excitatory pathways of which involve peripheral receptors, nerve cord, and a peripheral ganglion in each elytrum on the efferent side of the arc (Bonhomme, 1942; Nicol, 1954).

It has been observed that when a polynoid is transected, only the part posterior to the cut flashes, the anterior fragment remaining dark (Kutschera, 1909; Nicol, 1953). There is a seeming discrepancy between this restricted response, and the more widespread luminescence which attends tactile stimulation (Harvey, 1952). The observations could be explained if there existed some form of functional polarity in the nerve cord, and I have tested this possibility by means of electrical stimulation.

MATERIAL AND METHODS

Gattyana cirrosa was selected for study because it is large, tough, and sluggish. Specimens were anaesthetized in iso-osmotic $MgCl_2$, and heads and tails were cut off. The majority of elytra were removed, leaving a few at restricted levels, namely at the anterior end, middle of the body, and at the posterior end. The animal was slit longitudinally along the mid-dorsal surface, and the nerve cord was exposed. The preparation was then pinned out so that the residual elytra could be seen from above, and a pair of electrodes could be placed on the nerve cord.

Electrodes were platinum wire, insulated to near the tips. Electrical stimuli were condenser discharges or square wave pulses delivered from electronic apparatus. Stimulus strengths were kept near threshold.

OBSERVATIONS

When severed in two halves with a clean quick cut, *Gattyana* behaves like other polynoids, all elytra in the posterior fragment flashing, while elytra in the anterior fragment remain dark. Tactile stimulation causes all elytra to flash.

Electrodes were placed on the nerve cord at the following levels: anterior quarter, middle, and posterior quarter.

Electrodes in the anterior region

Results of stimulation were variable. Usually flashing occurred in the elytra at the anterior end, either on the first pulse or after a series of pulses. In these instances continued stimulation led to flashing in middle elytra. In other preparations stimulation caused middle and posterior elytra to flash (first pulse), followed by flashing in anterior elytra when stimulation was continued.

Electrodes in the middle of the body

Stimulation at this level evoked light in contiguous middle elytra and in elytra at the posterior end (first pulse). Protracted stimulation was followed by flashing in anterior elytra.

Electrodes in the posterior region

A single pulse caused posterior elytra to flash. A series of pulses evoked light in anterior elytra.

Protocols of two experiments are as follows:

(1) Preparation with two pairs of elytra, at anterior and posterior ends; electrodes in middle of worm.

1 shock: flash in posterior elytra. 24 shocks at 1/sec: flickering light in posterior elytra. Long bursts at 2, 3, 4 and 5/sec: flickering light in posterior elytra.

(2) Preparation with three pairs of elytra, anterior end, middle and posterior end; electrodes in anterior region.

Burst at 1/sec: flashing in anterior elytra, beginning with eighth shock. Burst at 1/sec: flashing in anterior elytra, beginning with fourth shock. Burst at 1/sec: flashing in anterior elytra, beginning with seventh shock.

Electrodes moved to posterior region.

Burst at 2/sec: flash on first shock in posterior elytra. Burst at 2/sec: flashing in posterior elytra beginning with first shock; flashing in middle elytra beginning with ninth shock.

CONCLUSIONS

In the anterior quarter of the body, excitation for luminescence tends to show preferential conduction anteriorly. Behind this level there is a preferential tendency towards posterior conduction. A single pulse in the posterior half of the body excites elytra behind the electrodes; with continued stimulation light spreads to elytra anterior to that level. Thus, there is evidence for functional polarity in the nerve cord, such that luminescent excitation is conducted with greater facility posteriorly over a large part of its length. The evidence is more equivocal for the anterior end: in the majority of instances conduction occurred with greater facility anteriorly. It would seem, therefore, that when a polynoid is cut in half, the brief excitation can travel only posteriorly. More protracted tactile stimulation generates longer excitation, overcoming resistance to anterior transmission.

REFERENCES

BONHOMME, C., 1942. Recherches sur l'histologie de l'appareil lumineux des Polynoinés. *Bull. Inst. océanogr., Monaco*, No. 823.

HARVEY, E. N., 1952. *Bioluminescence*. New York: Academic Press.

KUTSCHERA, F., 1909. Die Leuchtorgane von *Acholoe astericola* Clprd. *Zeit wiss. Zool.*, Bd. 92, pp. 75–102.

NICOL, J. A. C., 1953. Luminescence in polynoid worms. *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 65–84.

—— 1954. The nervous control of luminescent responses in polynoid worms. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 225–55.

—— 1957. Luminescence in polynoids. II. Different modes of response in the elytra. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 261–9.

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ON A NEW SPECIES OF SCYPHOMEDUSA, *ATOLLA VANHÖFFENI* N.SP.

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(Plate I and Text-fig. 1)

It seems to be the general opinion that there are only two species of *Atolla*, *A. wyvillei* Haeckel, with smooth marginal lappets, and *A. chuni* Vanhöffen in which the marginal lappets have warts.

But Vanhöffen (1902, p. 21, pl. v, figs. 27–29) described in some specimens organs which he thought might be excretory in function. These organs appeared as eight dark spots disposed radially on the subumbrellar walls of the stomach where the gastric cavity narrows towards the ostia. In these dark spots the ectoderm and endoderm cells are higher than those in the neighbouring areas, the ectoderm cells being more darkly pigmented than the endoderm cells. Sections do not show any passage through the mesogloea so that there are in fact no pores.

Maas (1903, p. 10, and 1904, p. 52) drew attention also to these spots and reproduced an excellent photograph of a specimen with them (Maas, 1903, pl. XII, fig. 108).

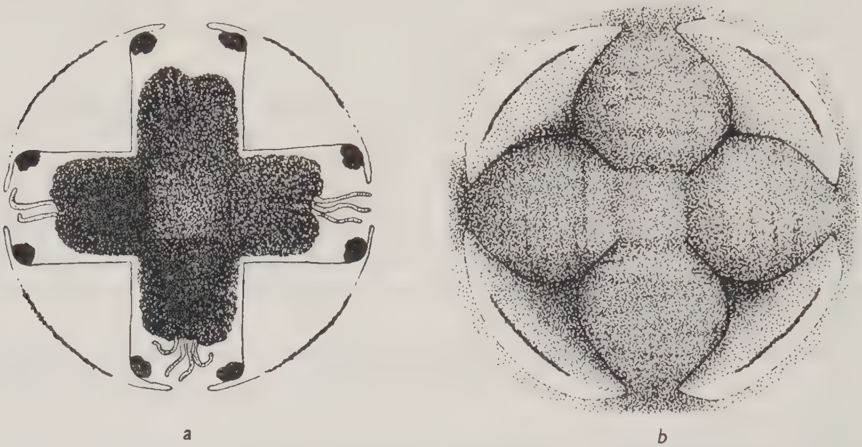
They were subsequently referred to by Stiasny (1934, p. 52) who saw them in some of the specimens in the 'Discovery' collections.

Recently I found two such specimens in a collection made on R.R.S *Discovery II* with a 2 m stramin ring trawl hauled obliquely from *ca.* 2400 m at 39° 38′ N., 11° 30′ W. on 26 February 1956 (Discovery Station 3370). The general appearance of these medusae was so different from that of all the other specimens of *Atolla* that I had seen that I suspected they might belong to a separate species. I was fortunate enough to find another such specimen in a collection made on R.V. *Sarsia* with a 2 m stramin ring trawl with 900 fathoms of wire out at 47° 03′ N., 5° 47′ W., on 3 July 1956. This specimen was in an excellent state of preservation, and with it was another well-preserved specimen of the same size without spots and with the pigmentation typical of *A. wyvillei*. It could be seen at once that they were so different that they must be specifically distinct (Pl. I).

Besides the difference of the presence or absence of the eight spots there are other quite obvious distinctions.

In the medusae with spots the basal attachment of the stomach forms a cross whose arms slope downwards towards the umbrella margin and are of approximately uniform width until they narrow suddenly to form the ostia into the gastrovascular sinus (Text-fig. 1*a*). In the typical *A. wyvillei* the basal attachment is quite different and has rather the form of a four-leaved clover (Text-fig. 1*b*).

In the medusa with spots the umbrella is almost entirely unpigmented, except for some faint pigmentation at the bases of the marginal tentacles; some clusters of minute spots on the ring muscle; and four curved lines of pigment along the bases of the four triangular septa. The gonads are yellowish brown on the exumbrellar side, and this pigmentation curves over the side of each gonad leaving a circular patch free of pigment on the subumbrellar side; one gonad is missing in this specimen (Pl. I).



Text-fig. 1. Exumbrellar view of the base of the stomach of *a*, *Atolla vanhöffeni* n.sp., and *b*, *A. wyvillei*. For clarity the buccal portion of the stomach has been omitted.

The stomach and the eight spots are intense blackish purple. The pigment stops short before the end of each arm of the cross leaving transparent spaces in which one or two of the pigmented gastric cirri may be seen. Beneath these transparent areas are the perradial pillars of jelly running down the outside of the buccal walls of the stomach.

This pigmentation is in marked contrast to the varying range of coloration to be found on the umbrella of *A. wyvillei*, in which the pigment is more reddish brown in colour. Even those specimens of *A. wyvillei*, in which only the stomach and gonads are pigmented (group I of Broch, 1913, p. 15), are immediately distinguishable by the shape of the base of the stomach and its reddish brown colour.

Vanhöffen (1902) described the dark spots in a general account of the histology of the genus *Atolla*, ascribing the character to no single species. Maas (1903, pl. I, fig. 4; pl. XII, fig. 108) figured the spots as *A. valdiviae*, but later (Maas, 1904, pl. V, fig. 38) as *A. bairdi*. Stiasny (1934) referred to them under a general account of *A. wyvillei*.

Since, therefore, the species with spots cannot be allocated to any known specific name I propose to call it *A. vanhöffeni* n.sp. in honour of Ernst Vanhöffen who was the first to draw attention to the occurrence of the spots.

I have so far seen three specimens: these were 15, 18 and 20 mm in diameter. Two of these had twenty tentacles, but the third was cut up for sectioning before they were counted. Two were females and one male. In the female shown in Pl. I each gonad had one very large egg about 1 mm in diameter with a few smaller eggs.

Pl. I gives a photograph of *A. vanhöffeni*, and, for comparison, the specimen of *A. wyvillei* found with it in the same collection.

I hope that I may find more specimens of *A. vanhöffeni*, when it may be possible to determine other specific characters. At any rate the following characters are quite sufficient for the identification of the species.

A. vanhöffeni	*A. wyvillei*
With eight pigment spots	Without pigment spots
Umbrella almost completely unpigmented	Umbrella with characteristic pigment pattern
Base of stomach in form of a cross	Base of stomach in form of a four-leaved clover
Colour of stomach and spots blackish purple	Colour of stomach and on umbrella brownish red

The largest specimen I have is 20 mm in diameter. It seems possible that *A. vanhöffeni* may not grow to so large a size as *A. wyvillei*, since all previous authors when recording specimens with spots have remarked that they are usually young specimens.

The specimen of *A. vanhöffeni* figured in Pl. I has been deposited in the British Museum (Natural History) and has been given the registration number B.M. 1957.1.30.1.

My thanks are due to Dr J. A. C. Nicol who preserved two of the specimens for me while on a cruise on R.R.S. *Discovery II*; to Captain C. A. Hoodless and the crew of R.V. *Sarsia* who collected the fine specimen on which the above account is based; and to Mr A. C. G. Best who took the photographs reproduced in Plate I.

SUMMARY

In 1902 Ernst Vanhöffen drew attention to the occurrence of eight dark pigment spots in *Atolla* which he thought might be excretory organs.

It is shown that these spots are a specific character occurring only in some specimens, and medusae with this character have been named *Atolla vanhöffeni* n.sp. This species can now be distinguished from *A. wyvillei* by other characters.

REFERENCES

Broch, Hjalmar, 1913. Scyphomedusae from the *Michael Sars* North Atlantic Deep-Sea Expedition 1910. *Rep. Sci. Res. 'M. Sars' Exped.*, Vol. 3, Part I, 20 pp.

Maas, Otto, 1903. Die Scyphomedusen der Siboga Expedition. *Siboga Exped.*, Monogr. XI, pp. 1–91.

—— 1904. Méduses provenant des Campagnes des Yachts *Hirondelle* et *Princesse-Alice* (1886–1903). *Rés. Camp. sci. Monaco*, Fasc. 28, pp. 1–71.

Stiasny, G. 1934. Scyphomedusae. '*Discovery*' *Rep.*, Vol. 8, pp. 329–96.

Vanhöffen, Ernst. 1902. Die acraspeden Medusen der deutschen Tiefsee-Expedition 1898–1899. *Wiss. Ergebn. 'Valdivia'*, Bd. 3, pp. 3–52.

Note added in proof

I have now found two more specimens of *A. vanhöffeni*, both collected from 47° 09′ N., 7° 38′ W. on 21 July 1955. One was a small specimen 8 mm in diameter: it had 18 marginal tentacles and developing female gonads. The other, beautifully preserved, was 25 mm in diameter; it had 20 marginal tentacles and was a female with one very large egg among other eggs in some of the gonads. This specimen differed from the description given above in that the muscles at the bases of the marginal tentacles were strongly pigmented.

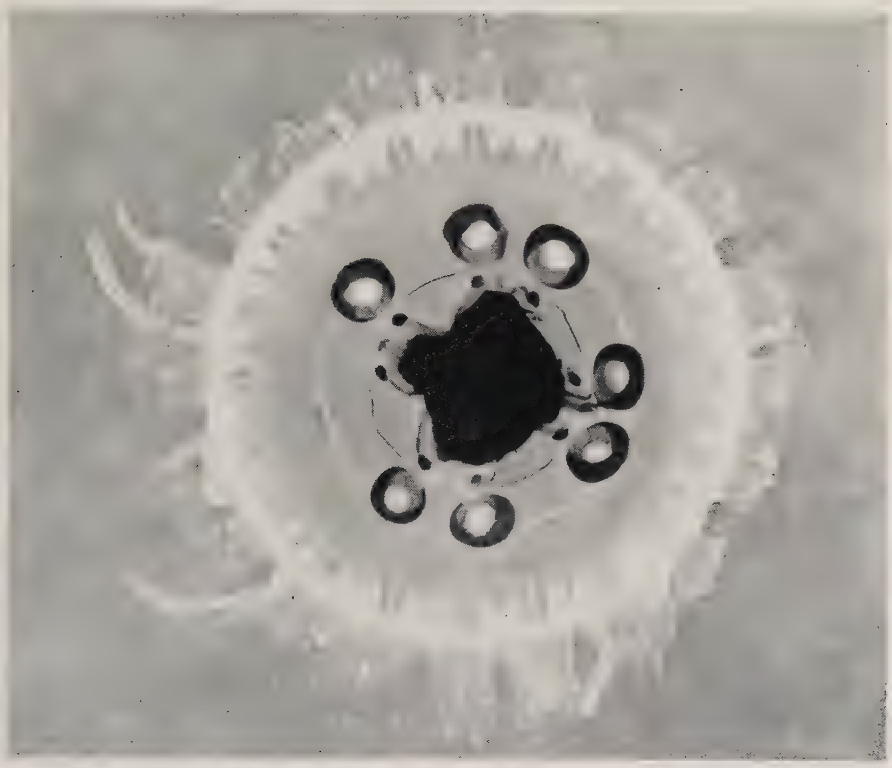
Through the kindness of Dr P. L. Kramp I have also been able to see several specimens of *A. vanhöffeni* picked out from the collections of *Atolla* from the Atlantic in the Universitetets Zoologiske Museum in Copenhagen by Dr Kay Petersen. The details are given below.

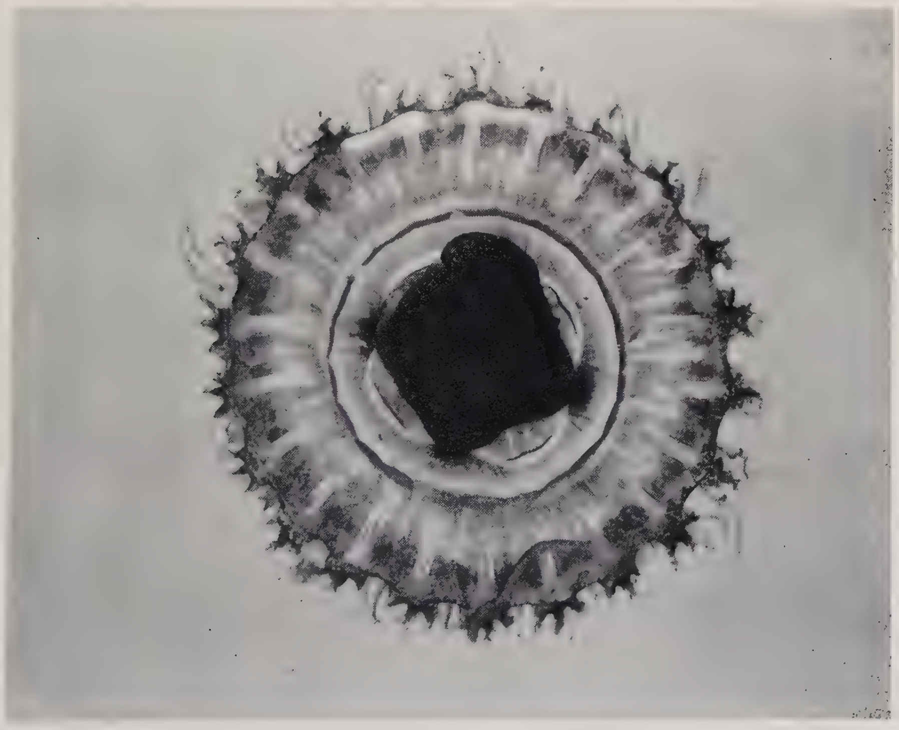
			Metres wire	Diameter (mm)	Sex	No. of tentacles
Thor						
3. ix. 1906	St. 180	48° 19′ N., 13° 53′ W.	—	12	♀	20
28. ii. 1909	St. 69	36° 13′ N., 9° 44′ W.	600	15	♀	20
4. iii. 1909	St. 71	39° 35′ N., 9° 45′ W.	600	13	♀	20
				12	♀	20
				11	♀	20
				10	? ♂	20
				8	?	Damaged
4. iii. 1909	St. 71	39° 35′ N., 9° 45′ W.	1600	29	♀	19
18. vi. 1910	St. 91	35° 53′ N., 7° 26′ W.	1600	10	♂	20
				10	No gonads	20
				9	No gonads	20
				8	♀	20
				7	? ♀	19
				6	♀	20
				9	♂	Damaged
9. ix. 1910	St. 232	36° 28′ N., 9° 06′ W.	2000	16	No gonads	20
				16	♀	20
				13	♂	20
Dana						
1. vii. 1931	St. 4206 II	53° 38′ N., 29° 41′ W.	600	25	♀	20

It will be noted that nearly all these specimens had 20 marginal tentacles, and none had more. I have now examined 21 complete specimens, 18 of which had 20 tentacles, two had 19, and one had 18. It seems probable that this is a constant character distinguishing the species from *A. wyvillei* in which the number of tentacles is typically 22.

EXPLANATION OF PLATE I

Above, *Atolla vanhöffeni* n.sp.; below *A. wyvillei*. Both specimens were collected from 47° 03′ N., 5° 47′ W. on 3 July 1956. They are photographs from the subumbrellar side and are both enlarged × *ca.* 4·5.





(*Facing p.* 280)

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THE BIOLOGY OF A COMMENSAL COPEPOD, *ASCIDICOLA ROSEA* THORELL, IN THE ASCIDIAN *CORELLA PARALLELOGRAMMA* (MÜLLER)

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(Text-figs. 1 and 2)

The considerable advantages offered by ascidians as hosts to small commensal crustaceans are clearly implied by the large number of ascidicolous copepods which have been described; and the attractions of this particular niche are further evidenced by the fact that these copepod mess-mates are of polyphyletic origin and cosmopolitan distribution.

The study of such associations, however, has been limited almost entirely to a bare note of their existence, although the papers of Canu (1892) and Chatton & Brément (1915) are partial exceptions.

That ascidians so frequently act as hosts is not in itself surprising. Their comparative immunity from predation, their maintenance of a feeding current and the capaciousness of many of their internal cavities afford protection, accessible food and a certain freedom of movement. Furthermore, the elaborate ascidian mechanism of food intake and concentration offers, in its several stages, different possibilities of utilization to copepod commensals of varying structure and mobility. Thus, in the pharynx alone, a commensal might exploit the particles entering the oral aperture; or it might seek these as, trapped in the moving sheets of mucus, they are slowly swept towards the dorsal lamina; or, yet again, the concentrated food string, *en route* to the oesophagus and stomach, might be the focus of attention. Encompassed, therefore, by this complex nutritional mechanism many variously adapted copepods find optimal living conditions.

In any attempt to shed some light on associations of this type, three conditions must be satisfied. The chosen copepod species must be of relatively large size, the ascidian concerned must possess an unusually transparent test, and observations must be made in a fairly large volume of undisturbed sea water to ensure normal behaviour on the part of the host. After a prolonged search for suitable material in Strangford Lough, Co. Down, it was found that the association between *Ascidicola rosea* Thorell and *Corella parallelogramma* (Müller) proved almost ideal. The female *Ascidicola rosea* attains a length of over 4 mm, while the glass-clear tunic of *Corella parallelogramma* is perhaps its most striking feature. A Perspex tank, coupled with a viewing system of lenses and ground-glass screens, with adequate background lighting, solved most of the problems of observation under nearly normal conditions.

GENERAL CONSIDERATIONS

C. parallelogramma, a solitary ascidian belonging to suborder Phlebobranchiata, occurs sparingly around the British coasts, being found attached to stones, weed, shells, etc., usually in clear offshore waters down to depths of 150 m (Berrill, 1950). Abroad, its range includes a large part of the Scandinavian coast and the Mediterranean. It attains a length of some 5 cm, large specimens being easily dredged in about 12 m in Strangford Lough, where the species is abundant on suitable ground, especially during the autumn months. As well as *Ascidicola rosea*, *Corella* is known to harbour several other ascidicolous copepods—*Notodelphys agilis* Thorell, *N. caerulea* Thorell, *Doropygus porcicauda* Brady, *D. pulex* Thorell, *Pachypygus gibber* Thorell, and the cyclopoids *Ascomyzon lilljeborgi* Thorell and *Lichomolgus albens* Thorell, the last-named sometimes cohabiting with *Ascidicola*. At the beginning of October, *A. rosea* was present in about 25 % of *Corella* examined; three weeks later 60 % of the hosts were infected.

Ascidicola rosea is usually classified as the sole member of the family Ascidicolidae, suborder Notodelphyoida. In a recent paper, however, Lang (1948) has suggested that this very heterogeneous suborder should be split up, in which case the Ascidicolidae, along with several other notodelphyoid families, should be regarded as a special tribe of gnathostomatous cyclopoids—a taxonomic realignment which has much to recommend it. This copepod has been reported from several localities around Britain and Ireland, while abroad it is found on the Norwegian coast as well as in the Baltic and Mediterranean. In addition to *Corella* its hosts include *Halocynthia papillosa* (L.), *Ascidia mentula* (Müller), *A. obliqua* Alder, *Ascidiella aspersa* (Müller), *A. opalina* M'Gill, *Ciona intestinalis* (L) and *Phallusia* sp. I have also obtained a single female specimen from *Pyura squamulosa* (Alder).

During the winter months, the *Corella* population of Strangford undergoes a very marked diminution. Since the commensal breeds throughout the year, the copepodids must therefore seek alternative hosts during the colder months in order to complete their development. *Ascidiella aspersa*, which remains plentiful during the critical period, is a frequent choice, and it seems likely that it is this species which carries the main bulk of the overwintering *Ascidicola* population.

Until recently, the genus *Ascidicola* was thought to be monotypic and restricted to the northern hemisphere, but Lang (1949) has described a second, closely allied species, *A. aculeoretusa*, from *Pyura georgiana* Mchlsn. taken in 250 m off South Georgia.

POSITION AND FEEDING ACTIVITIES OF THE COPEPOD

Schellenberg (1921) summarized in tabular form the then available information regarding the site occupied by *Ascidicola rosea* within its various ascidian hosts, and the occasional records of the past thirty years have added to his list little of material value. In most cases, it is reported to occur in the pharynx, although a minority of observers have collected specimens from the stomach and intestine. Examination of infected living *Corella*, however, leaves little doubt that (in this host at least) *Ascidicola's* normal 'operational base' is neither pharynx nor stomach, still less the intestine, but the oesophagus. The stomach is entered only in certain circumstances, while excursions into the pharynx proper are rare indeed. On only one occasion, in fact, was *Ascidicola* observed to cross the pharyngeal wall.

The preference shown for the oesophageal region is explained by the proximity of the food string descending the dorsal lamina, for the particles entrapped on it appear to provide this copepod with its only source of nourishment. During active feeding, *Ascidicola* grasps the mucus cord with the four anterior pairs of pereiopods and climbs up it for a short distance, feeding intermittently. Although as much as two-thirds (or, exceptionally, the whole) of the body may project head first into the pharynx, the slow downward movement of the food string as it passes into the ascidian's stomach tends continually to draw the copepod back into the oesophagus. After sinking a short distance in this way, *Ascidicola* adjusts its position by another climbing movement. The initiation of a fresh ascent often appears to follow contact between the urosome of the passively descending commensal and the curving wall of the oesophagus where the latter narrows before entering the stomach. During its ascent, *Ascidicola* may climb in a straight path or adopt an irregular side to side course. Not infrequently a complete spiral is achieved, although this is probably due in part to the twisting of the cord itself. While on the food string, the copepod twitches its antennules intermittently and various unidentifiable movements of the antennae and mouthparts can be seen. Occasionally it may tug at the food string with a vigorous movement of the head, sometimes producing a noticeable kink in the mucus cord. Whether this action is designed to remove an attractive food particle or to accelerate the downward passage of the still unexplored upper portion of the string, it is difficult to say. Evidence in support of the latter interpretation may be provided by the fact that this movement generally takes place when the food string is thin and poorly developed. Feeding activities may cease for short periods, the copepod withdrawing its head and anterior segments from contact with the cord and remaining motionless in an attitude somewhat resembling that of a resting sphingid moth larva. Climbing activity appears to be greatly reduced in the presence of a very thick food string, the commensal remaining almost motionless and pressed against the oesophageal wall.

When active feeding is not taking place, *Ascidicola* lies cradled in the oesophageal bend, usually with the ventral surface uppermost. Since, in this position, the food string is passing in close proximity to the mouthparts, it is possible that some feeding still occurs. Close observation, however, indicates that this must be on a reduced scale, if in fact it takes place at all.

Feeding activity appears to be of a non-cyclical nature. Certainly no daily periodicity is involved, since copepods may still be seen on the food string several hours after darkness has fallen as well as during the day. The periods of quiescence, therefore, spent cradled in the oesophageal curve, in most cases probably follow optimal feeding on a rich food string, thus establishing an intimate link with the trophic activity of the host.

Under laboratory conditions, *A. rosea* will often adopt a third position, lying in the oesophagus with its head towards the ascidian's stomach. When in this attitude, the copepod may bend the terminal segment of the urosome upwards to an angle of 45°, in this way perhaps achieving a braking effect against the ciliary current of the oesophagus. This inverted position is very frequently associated with a poorly developed or completely interrupted food string, and suggests that the commensal turns to follow the final truly nutritive section of the cord as it passes into the digestive regions of the host's canal. Often this attitude is maintained for long periods, *Ascidicola* sometimes seeming to grasp and halt the receding portion of rich food string before it slowly vanishes into the host's stomach. In this way the stomach may be actually entered for varying periods of time, although in the natural state it is probably seldom necessary to seek food in this region. An interruption of the host's normal feeding pattern, consequent upon its capture, may well account for some of the reported instances when *Ascidicola* has been found in an ascidian's stomach. (There is, however, some slight evidence that rather more time may be spent in this region when the host concerned is *A. aspersa*.) Only twice during the present study have copepods been seen in the intestine and twice in the rectum.

Two occasions on which the commensals were observed to enter the stomach are worthy of record. Once, after several ascents, the copepod remained quiescent on the descending food string and was eventually drawn, tail first, into the stomach. Some minutes later, after several exploratory movements, it emerged head foremost and once again mounted the food string. In another instance, following the introduction of a second *Ascidicola* which became trapped on the descending cord, the rightful occupant turned completely round and crawled into the stomach, followed passively by the immobilized specimen. A similar turning reaction sometimes takes place if an abrupt stimulus is applied—for example, focusing a concentrated spot of light on the copepod.

Several hitherto puzzling features of *A. rosea's* anatomy become explicable in the light of its intimate association with the food string. Thus Sars (1921) described the penultimate abdominal segment as 'having the ventral part of the hind edge remarkably thickened and densely clothed with small pricks'—a feature which was also noticed by Brady (1878). These 'small pricks' are in fact tiny sharply pointed spines, and since the region in which they occur is pressed closely against the mucus cord there can be little doubt that they aid in maintaining a firm grip, functioning, in fact, rather like a crampon (Fig. 1).

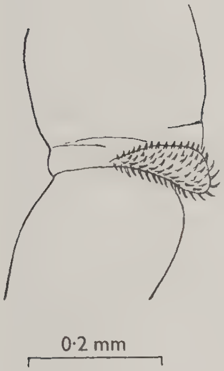

Fig. 1

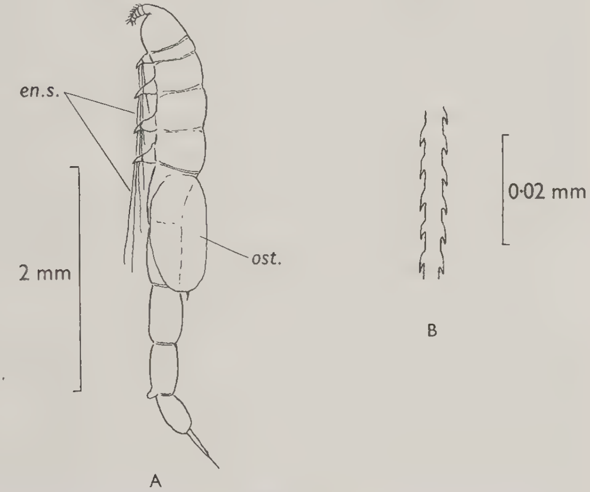

Fig. 2

Fig. 1. Distal part of penultimate segment of female *Ascidicola rosea* to show the spinous pad. The proximal part of the anal segment is also shown.

Fig. 2. A, female *Ascidicola rosea* in lateral view to show the long endopodal setae (*en.s.*) and the left oostegite (*ost.*). B, a small portion of an endopodal seta, showing the spines with which it is beset.

During a climb, it is occasionally noticeable that the anterior urosome segments will extend telescopically, carrying the body upward, while the posterior two remain temporarily motionless in close contact with the food string, thus providing a point of leverage. The above-mentioned bending of the terminal urosome segment is also apparent during some climbs, although its object in this case is clearly to bring the spinous pad on the penultimate segment into more effective play.

The extraordinarily long setae carried by the endopodites of the four anterior pereiopods are also of interest in a similar connexion. In life, their tips are directed posteriorly (Fig. 2A) and the entire length of each seta is applied to the main axis of the food string. In spite of Sars's assertion (1921) that these setae are quite smooth, examination under high power reveals them as beset with minute spines (Fig. 2B). It is possible that these spines, by engaging in the food string, likewise represent an adaptation to assist in maintaining a secure grip while in a vertical position on the cord.

RELATIONS WITH THE HOST

Although Lang (1948) records single specimens of *Corella parallelogramma* as harbouring up to five females of *Ascidicola rosea*, the rule in dredged ascidians from Strangford Lough seems to be one copepod per host. Occasionally, however, a very young female will be found with an adult, the former occupying a more anterior site on the food string. As this rather large commensal appears to depend entirely on food particles removed from the string, and as its predilection for the oesophageal region is very marked, it would be interesting to know the exact sites occupied by Lang's specimens if the latter were fully adult. One can only conclude that some might well have been crowded into non-optimal positions within their hosts. In many ascidicolous copepods, of course, more than one individual may frequently be found inhabiting a single ascidian. Thus several *Notodelphys allmani* Thorell are of common occurrence in even rather small specimens of *Ascidiella aspersa*—but in this case the commensals range freely throughout the pharynx and are not virtually restricted to the neighbourhood of the food string.

Infected *Corella* appear to be perfectly healthy, feeding and spawning quite normally, and with a survival rate, under laboratory conditions, comparable with that of uninfected specimens. The available evidence, then, suggests that *Ascidicola* deprives its host only of an insignificant fraction of its food, and certainly does not appear to injure it in any other way. Indeed, even some of the food particles filched from the string might subsequently become available to the host in the form of the copepod's faecal material, since organic debris is, of course, utilized by ascidians as well as living micro-organisms. While we are therefore faced with the usual, if artificial, difficulty of deciding on the precise ecological status of *Ascidicola*, its description as an endocommensal would seem to fit the known facts accurately enough.

REPRODUCTION

A. rosea carries its pink-coloured eggs in two thin oval masses protected by the dorsally situated lamellate oostegites (see Fig. 2A). These structures were regarded by Canu (1892) as the enlarged and modified fifth pair of pereiopods, although his interpretation was later disputed by Chatton & Brément (1915), who maintained that they could equally be akin to the alate processes of the notopterophorids. Their exact nature, therefore, may still be regarded as *sub judice*. Each egg-mass is slightly concave ventrally, being moulded to the contours of the body, and each contains approximately the same number of eggs. The actual egg number, however, may vary within rather wide limits. This variability may be governed, as Marshall & Orr (1952) have shown for *Calanus finmarchicus* (Gunnerus), by the food supply available to the parent, or alternatively may be correlated with the decreasing fertility of a female which has already, perhaps, produced several broods. Whatever the reason, an egg-mass may contain as few as 20 or as many as 70 eggs. Individual eggs measure 0·13–0·14 mm in diameter.

The male, which attains little more than 1 mm in length, lacks the obviously specialized features of the mature female and is adapted for an active free-swimming existence. As one might expect, it is encountered much less frequently. Sars (1921) mentions having found only a single specimen, dredged in the free condition. None have been seen in ascidians from the Strangford area and it can probably be assumed that its occurrence as an adult in the host is in the nature of a fleeting visit only, during which mating takes place. The possibility, however, cannot be excluded that fertilization is performed by a male which had developed within the same host as a young female and which has subsequently left the ascidian, since Marshall & Orr (1955) have shown that in *Calanus* fertilization takes place at a relatively early stage of ovarian development. On the other hand, the fact that not one adult male has been detected within *Corella* during the Strangford investigation renders this a less likely explanation.

Perhaps the most interesting problem which arose during the study of this association concerned the mode of exit from the host of the copepod's young stages. Short of rupturing both mantle and tunic—a task manifestly beyond the powers of either eggs or nauplii—and in the absence of evidence that the peribranchial slits were utilized, the only available escape routes would be via either the oral or the atrial siphons. If the eggs hatched *in situ* on the parent copepod and the nauplii attempted to reach the outside world via the oral route, they would encounter not only the force of the inhalant current but the dangers attendant upon a journey through the mucus-lined pharynx—a structure specifically evolved for the capture of micro-organisms. To traverse the entire length of the food-laden alimentary canal, however, would involve an equally hazardous passage for these fragile forms. It seemed probable, therefore, that egress took place in the egg stage. This would, however, need very accurate timing, since to shed relatively large demersal eggs from the protection of the host long before hatching would unnecessarily present hostages to fortune in view of the ubiquity of benthic scavengers.

In the account which follows, although the initial part of the process has not been observed in detail, due largely to the opacity of the ascidian's stomach wall, it can be inferred beyond any reasonable doubt to take place as described. Some time before the eggs are due to hatch, the female leaves her position in the oesophagus and enters the stomach. Here the ripe egg-masses are detached from beneath the oostegites. (This occurs quite regularly in females extracted from the host.) Shortly after being deposited in the stomach, the inner membrane of each egg, with its contained nauplius, becomes free, leaving the cluster of adherent outer membranes to remain as a honey-combed ghost-like replica of the original egg-mass—again a noticeable occurrence with ripe isolated ovisacs. From the host's stomach, the nauplii—still sheathed in their protective inner membranes—are carried in small or large groups through the intestine and up the rectum, in which region they can be clearly seen provided only a small quantity of food material is present in the host's gut. In one instance a cluster of some 30 eggs took 30 min to traverse the last 8 mm of the rectum. As the anus is reached, and the full strength of the exhalant current encountered, final rupture of the delicate enveloping membrane takes place and the freed nauplii are expelled with almost explosive force through the atrial siphon. In a few of the hatchings observed, a group of eggs became lodged in a small terminal 'pocket' of the host's rectum, where they remained for some hours, but whether this would occur in ascidians with a full gut is difficult to determine. Meanwhile the female copepod has re-entered the oesophagus, and a new batch of eggs has, in most cases, been squeezed into the space beneath her oostegites. Eighteen days elapsed between successive hatchings of nauplii from one female observed in the laboratory.

This method of egg dispersal via the food canal of the host has not, to my knowledge, been described in any other copepod—though it is quite possibly the usual method for many of the species living in simple ascidians. It is, in its way, quite as remarkable as the egg-depositing migration of another ascidicolous form, *Enterocolides ecaudatus* Chatton & Brément, in which the mature female burrows up from the depths of its compound ascidian host to leave its egg-masses at the surface of the colony (Chatton & Harant, 1924). It is interesting also to observe how the presence of the inner egg membrane (a common feature in many free-living copepods, as shown by Marshall & Orr, 1954) is here of vital adaptive significance, serving as it does to protect the fragile nauplius during its passage through the host's gut.

Although only a subsidiary part of the present study, the various larval stages of *Ascidicola* perhaps merit the following brief notes, though it must be emphasized that the times given are approximate only. There are four clearly defined naupliar stages. The first-stage nauplius measures between 0·191 and 0·197 mm and swims with the usual jerky movement, exhibiting a marked preference for certain light intensities. After about 21 h, at a laboratory water temperature varying between 10·9° and 16·9° C, it moults to the second nauplius, which measures 0·206 mm. Again after approximately 21 h, the third nauplius appears, measuring 0·211 mm. 24 h later, the final nauplius stage is found, which attains 0·224–0·229 mm in length. This instar persists for about 31 h before the moult takes place to the first copepodid, which measures 0·349 mm and possesses six segments in all. At this stage the three anterior pairs of swimming legs are developed, though the third pair are still somewhat rudimentary. After a further 54 h, the second copepodid appears. This shows an increase in length to 0·369 mm, and possesses seven segments as well as a fuller development of the third pair of legs. After swimming freely for some time it displays a different type of behaviour, seeking the bottom of the dish and remaining quiescent for lengthy periods. If a *Corella* is now placed in the same vessel, on subsequent examination it will be seen to contain copepodids scrambling about on the food string in the oesophageal region. Although fuller confirmation is desirable that the second copepodid represents the infective phase, there is little doubt that the developmental pattern will prove similar to that of *Enterocola fulgens* van Beneden (see Canu, 1892), in which further moults to the adult female stage occur within the host after entry by the second copepodid. It seems highly probable that a period spent in the ascidian is obligatory for all copepodids, male as well as female, if one can judge by the universal demise of copepodids not provided with a potential host. It must be admitted that these stages have not been seen often in *Corella*, but as they are so small they could very easily be overlooked, even when specifically sought.

It is hoped at some later date to investigate the nature of the attraction which the host ascidian apparently exerts over the second-stage copepodid. It seems unlikely that intake by chance alone should exercise a controlling influence on a life cycle so perfectly attuned in all other respects to the survival requirements of this specialized commensal.

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SUMMARY

A study has been made of the feeding activities and reproductive behaviour of the copepod *Ascidicola rosea* within one of its ascidian hosts, *Corella parallelogramma*.

Ascidicola rosea feeds on particles which it removes from the food string as the latter is passing through the oesophagus of the host. When active feeding is not taking place, the copepod remains quiescent in the oesophageal bend. An inverted position within the oesophagus is sometimes adopted; this is generally associated with a meagre development of the food string. Certain peculiar structural features—notably the spinous pad on the penultimate segment and the long endopodal setae—are considered to be adaptations which assist the copepod while clinging to the food string.

The eggs of *A. rosea* are deposited in the ascidian's stomach and the nauplii pass through the host's alimentary canal still enveloped by the inner egg membrane. On reaching the anus, hatching takes place and the nauplii are expelled from the atrial siphon. There are four naupliar and two copepodid stages. Free-swimming life lasts for about 6 days.

REFERENCES

Berrill, N. J., 1950. *The Tunicata, with an Account of the British Species.* London: Ray Soc.

Brady, G. S., 1878. *A Monograph of the Free and Semi-Parasitic Copepoda of the British Islands*, Vol. 1. London: Ray Soc.

Canu, E., 1892. Les copépodes du Boulonnais. *Trav. Sta. zool. Wimereux*, Vol. 6, 354 pp.

Chatton, E. & Brément, E., 1915. Les oostégites, les ptérostégites et la cavité incubatrice des Ascidicolidae (Copépodes); développement, homologies, valeur phylogénitique et taxonomique. *Bull. Soc. zool. Fr.*, Vol. 40, pp. 143–55.

Chatton, E. & Harant, H., 1924. *Lequerrea perezi* n.g., n.sp., enterocolien parasite d'une ascidie simple. *Bull. Soc. zool. Fr.*, Vol. 49, pp. 24–30.

Lang, K., 1948. Copepoda 'Notodelphyoida' from the Swedish west-coast with an outline on the systematics of the copepods. *Ark. Zool.*, Bd. 40A, pt. 14, 36 pp.

—— 1949. On a new copepod family related to Notodelphyidae and on two new copepod species from South-Georgia. *Ark. Zool.*, Bd. 42B, pt. 4, 7 pp.

Marshall, S. M. & Orr, A. P., 1952. On the biology of *Calanus finmarchicus*. VII. Factors affecting egg production. *J. mar. biol. Ass. U.K.*, Vol. 30, pp. 527–47.

—— 1954. Hatching in *Calanus finmarchicus* and some other copepods. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 393–401.

—— 1955. *The Biology of a Marine Copepod,* Calanus finmarchicus *(Gunnerus)*. Edinburgh.

Sars, G. O., 1921. *An Account of the Crustacea of Norway*, Vol. 8. Copepoda Monstrilloida and Notodelphyoida.

Schellenberg, A., 1921. Neue Notodelphyiden des Berliner und Hamburger Museums. II. Teil. *Mitt. zool. Mus. Berl.*, Bd. 10, pp. 277–98.

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ON THE HORMONAL INHIBITION OF MOULTING IN DECAPOD CRUSTACEA

II. THE TERMINAL ANECDYSIS IN CRABS

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(Text-figs. 1–5)

When a crab moults it usually, though not invariably, increases in volume and in linear dimensions. The interval between successive moults or ecdyses frequently varies with the temperature and becomes greater as the individual grows larger. An exception is to be found in some crabs which have a regular moulting season, moulting perhaps once or twice a year. In some species at least the cycle of growth and moulting terminates with a final terminal anecdysis, beyond which the crab never progresses. This lays an upper limit to the size which the animal may attain. Teissier (1935) was, so far as I am aware, the first to draw attention to this phenomenon, whereby a crab—in Teissier's investigations the spider crab, *Maia squinado*—never moults again once it has entered upon this final instar. The occurrence of this terminal anecdysis has been demonstrated in the grapsoid crab *Pachygrapsus crassipes* (by Hiatt, 1948), the female blue crab *Callinectes sapidus*, and the green shore crab *Carcinus maenas* (by Carlisle, unpublished); it may be inferred from the somewhat scanty population statistics for other species of crabs, e.g. *Portunus depurator*, which have a definite size limit. The terminal anecdysis is, however, unknown in prawns which may go on moulting and increasing in size until they are overtaken by death, and the same is evidently true of the lobster, *Homarus gammarus* (L.) and the edible crab, *Cancer pagurus*, which may, apparently, increase in size indefinitely; reports of 'giant' crabs and 'giant' lobsters are not infrequent in the lay press and in the fishing periodicals.

In this paper the nomenclature of the moult cycle proposed by Carlisle & Dohrn (1953) will be adopted with addition of the expression 'terminal anecdysis'. Briefly the stages are as follows.

Proecdysis: a period of preparation for the moult, often accompanied by a raised blood-calcium level.
Ecdysis: the act of casting the shell or moulting.
Metecdysis: a period of recovery from the moult, when the animal is still partially soft but is rapidly hardening the new exoskeleton.
Diecdysis: a brief period between the end of one metecdysis and the beginning of the next proecdysis.

Anecdysis: a long period between the end of a metecdysis and the beginning of the succeeding proecdysis; this period often separates groups of moults which are themselves separated by diecdyses, but in species which moult seasonally, it is the normal stage separating two ecdyses.

Diecdysis and anecdysis are often both called intermoult periods, but their endocrine basis appears to be different (see Knowles & Carlisle, 1956) so that separate names are required for them. The term 'intermoult period', moreover has often been used to indicate the total period between two moults, including metecdysis and proecdysis. 'Terminal anecdysis' is a period at the end of the life of a crab when the physiological condition resembles a normal anecdysis, but the animal is incapable of further moulting. It will be noticed that this condition is strictly an anecdysis, but it can hardly be called an intermoult, for it does not lie between two moults. Some differences between animals in non-terminal and in terminal anecdysis will become apparent during the course of this paper.

The problem which has been investigated in the work now to be described is that of the failure to undergo further moults once terminal anecdysis is reached. The approach has been endocrinological.

MATERIALS AND METHODS

THE CRABS

Two crabs have been the subject of these investigations, the oxyrhynchan *Maia squinado*, the spider crab, and the brachyrhynchan *Carcinus maenas*, the green shore crab. All crabs were collected in the Plymouth area. *Carcinus* of the Plymouth population are biometrically and physiologically distinct from two races which occur on the opposite coast of the English Channel and it is probable that other local populations are likewise distinct (see Démeusy & Veillet, 1953; Démeusy, 1953; Carlisle, 1955); numerical data and dimensions refer specifically to the Plymouth population unless some other locality is mentioned.

Maia squinado has been the subject of intensive biometrical study by Teissier (1934–55), who has shown that it never moults again after the moult of puberty. Sexual maturity is achieved at this moult; copulation takes place while the female is still soft after the moult and is not possible thereafter. No further growth takes place. The terminal anecdysis, therefore, is the anecdysis succeeding upon the attainment of sexual maturity. It follows that female *Maia* can only breed once. The earlier moults are separated by long intervals, and the intermoult period appears to be of the nature of an anecdysis, rather than a diecdysis. So far as we are aware at present *Maia* only moults during late July and early August (always excluding the very young crabs). As a seasonal moulter, therefore, its intermoult period is almost certainly an anecdysis.

Carcinus maenas on the contrary moults throughout the greater part of the year, refraining from doing so only during the coldest part of the winter, January to March. The moults are separated by diecdyses during the summer and autumn; the only anecdysis is that during these three cold months. I have shown (Carlisle, 1954, 1955, and unpublished data) that the endocrine control of these two types of intermoult period is somewhat different. A detailed biological account of a brachyrhynchan crab, *Pachygrapsus crassipes*, whose moulting cycle follows the same pattern is given by Hiatt (1948). Like *Pachygrapsus*, *Carcinus maenas* ends its life in anecdysis, a terminal anecdysis after which it never moults again. In the Plymouth population almost all *Carcinus* over 70 mm carapace breadth and all over 75 mm are in the terminal anecdysis. The largest of the males are regularly 86 mm in carapace breadth, never in my experience exceeding this figure by even 1 mm. The females are smaller than the males. Analysis of the dimensions of egg-bearing females reveals a bimodal curve of size. I take this to indicate that there are two age-groups breeding each year. In the laboratory I have reared a crab caught when egg-bearing, with a carapace breadth of 23 mm, through four moults to a second breeding and egg-bearing a year later when the carapace breadth was 51 mm. After egg-bearing it moulted once more to a carapace breadth of 63 mm when it entered terminal anecdysis. The moult of puberty takes place in *Carcinus* at about 16 mm carapace breadth (Cornubert, Démeusy & Veillet, 1952), and the animal undergoes about ten more moults before entering on the terminal anecdysis. The contrast with *Maia* is obvious. It will be shown in the course of this paper that the endocrine basis of terminal anecdysis differs profoundly in the two species of crabs.

THE ENDOCRINE ORGANS

The X-organ-sinus gland complex of the eyestalk has been described in various species of decapod Crustacea by Bliss, Durand & Welsh (1954), Passano (1953) and Carlisle (1953) among others. It has repeatedly been shown to be the source of a moult inhibiting hormone (see especially Passano, 1953), but it does not apparently secrete a moult accelerating hormone, such as is found in the natantian decapods (see discussion in Knowles & Carlisle, 1956).

The Y-organ described by Gabe (1953) has been shown by Echalier (1954, 1955) to secrete a moult-promoting hormone. It is possible that the moult-inhibiting hormone of the eyestalk acts not on the tissues, but on the Y-organ, to restrain it from producing the moult-promoting hormone.

In this study attention was directed to these two endocrine complexes.

All extracts of the endocrine organs have been made in distilled water, by grinding up the fresh organ with fine silica sand in a fused alumina mortar and pestle. The resulting suspension was then either filtered or centrifuged, before it was injected intramuscularly.

Previous studies have shown that eyestalk removal and removal of the

X-organ-sinus gland complex have identical effects on moulting (see especially Passano, 1953). As far as the moult-inhibiting hormone of this complex is concerned eyestalk removal is simply a cruder way of effecting ablation of the X-organ-sinus gland complex. Both types of operation have been performed in this investigation, and the results have been strictly equivalent. Eyestalk removal has been performed by cutting through the base of the stalk with fine pointed scissors. One eyestalk has been removed at a time, the other left to the next day in order to reduce the mortality. Removal of the X-organ-sinus gland complex was effected by the method described by Passano (1953), with the obvious modifications necessitated by the slightly different anatomy of the species used. The Y-organ was removed by the method of Echalier (1954).

ASSAY OF EXTRACTS

All assays were performed upon non-egg-bearing female *Leander serratus* of the size-range 55–70 mm overall length. Individuals were chosen which were in diecdysis at the time of injection. Intact individuals were employed and each was injected with a single dose of 0·15 ml. of extract or control saline. Each dose level was administered to twenty prawns and the death-rate during the assay was of the order of 4%. In no group was more than a single test animal lost during the course of an assay. Five days after the injection the prawns were examined under the binocular microscope to determine what proportion of each group had begun proecdysis. The moult-inhibiting hormone of the X-organ-sinus gland complex inhibits the onset of proecdysis while the moult-promoting hormone of the Y-organ promotes its onset. The statistical analysis followed the recommendations of Finney (1952). Graphical illustrations of assays (Figs. 1, 2, 4 and 5) will explain their layout. No units of activity are established: all assays are strictly comparative, devoted to comparing the titre of hormone in the glands in one stage of life with that in another.

RESULTS

Carcinus maenas

Moult-inhibiting hormone

A comparative assay of sinus glands taken from the eyestalks of male *Carcinus* in the terminal and in earlier anecdyses is illustrated in Fig. 1. It will be seen that the titre of moult-inhibiting hormone per gland is roughly four times greater in the sinus glands of crabs taken during the terminal anecdysis than during earlier anecdyses. Statistical analysis of the data gives the ratio between the effectiveness of the two as 4·25 with 1% fiducial limits at 3·44 and 5·79. It is to be noted that crabs in the terminal anecdysis are larger than younger crabs, so if the data are presented in terms of mg of glandular tissue rather than numbers of glands, the ratio is diminished. Weight for weight the sinus glands of the older crabs are 2·68 times more effective than those of the younger crabs in preventing the initiation of proecdysis in *Leander serratus*, and the 1 % fiducial limits are 1·38 and 3·64. On either basis then, the sinus gland contains more moult-inhibiting hormone during the terminal anecdysis than during earlier anecdyses.

The difference is even greater between the terminal anecdysis and diecdysis. Here the ratio of titres of moult-inhibiting hormone (on a weight for weight basis) is 7·92 with 1 % fiducial limits at 6·75 and 9·37. This emphasizes once more that during diecdysis there is a lesser production of moult-inhibiting hormone by the eyestalk than during anecdysis; the endocrine status is different.

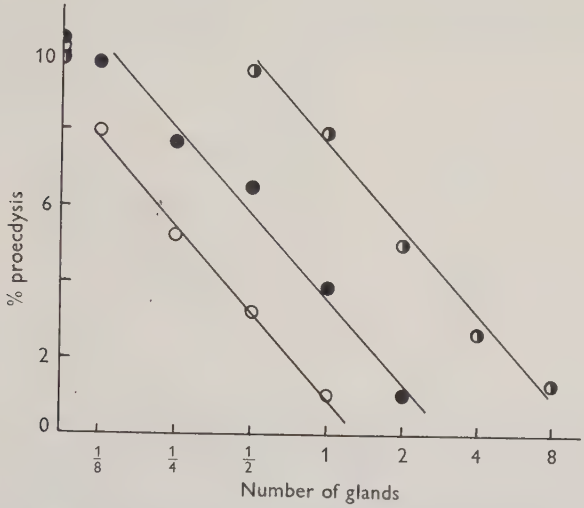

Fig. 1. Pooled results from a number of assays of the moult-inhibiting hormone of the sinus gland of *Carcinus* on *Leander*. The abscissa gives the number of glands supplying the extract which was injected into each individual; the ordinate gives the percentage of *Leander* which were found to be in proecdysis 5 days after the injections were made. All the *Leander* were in diecdysis at the beginning of the assay. Donors in terminal anecdysis—open circles; donors in non-terminal anecdysis—black circles; donors in diecdysis—half-black circles.

The high titre of moult-inhibiting hormone present in the sinus glands of *Carcinus* during the terminal anecdysis suggests that it may be part of the cause of this phenomenon. Removal, therefore, of the source of the hormone should test this hypothesis. This may be performed most simply by removing the whole eyestalk or by removing the X-organ-sinus gland complex, leaving the visual centres of the eyestalk intact. Both operations have led to the same result. All crabs in terminal anecdysis so operated have immediately entered proecdysis, as evinced by the heightened blood-calcium level and the modifications of the structure of the shell and cuticle. Eventually all which have survived long enough have moulted. The numbers are summarized in Table 1. During twelve months following the operation one crab whose eyestalks had been removed and two from which the X-organ-sinus gland complex had been removed moulted three times, while one whose eyestalks had been removed moulted four times. This last crab at death had a carapace breadth of 132 mm, compared with the largest specimens caught wild in Plymouth which regularly have a carapace breadth of 86 mm, and one large specimen from the Scilly Islands which measured 92 mm. It is noteworthy that *Carcinus* in terminal anecdysis have never survived more than 7 months in the laboratory.

There can be little doubt that the moult-inhibiting hormone of the eyestalk in *C. maenas* is a major agent in preventing ecdysis once the crab enters upon its terminal anecdysis. To stimulate further moulting it is merely necessary to remove the gland system responsible for secreting this hormone. It has earlier been shown (Brown & Cunningham, 1939; Passano, 1953) that injection of this hormone, or implantation of the gland, is sufficient to prevent the onset of proecdysis and subsequent ecdysis in crabs which have been deprived of the X-organ-sinus gland complex or of the eyestalks.

TABLE 1. MOULTING IN *CARCINUS* PRODUCED BY EYESTALK OPERATIONS IN ANIMALS IN TERMINAL ANECDYSIS

No. operated ...	Blank operation 100		Eyestalks removed 100		X-organ-sinus gland complex removed 100	
Week	Dead	Moulted	Dead	Moulted	Dead	Moulted
1	16	—	14	—	23	—
2	2	—	4	—	2	—
3	—	—	2	—	3	—
4	1	—	3	1	—	4
5	2	—	—	7	3	8
6	4	—	2	13	—	9
7	—	—	5	29	2	23
8	2	—	—	16	1	19
Total	27	0	30	66	34	63

The blank operation consisted in exposing the visual ganglia of the eyestalk, without damaging the endocrine complex; in a number of animals the visual centres themselves were damaged. All animals were males in terminal anecdysis. During the first 8 weeks of the experiment none moulted twice, thereafter a number of the animals moulted a second time. The dead recorded in the table are those which died without moulting first: a small number died after starting or completing the moult; these are counted among the animals which moulted.

The Y-organ

It is not known for certain whether the moult-inhibiting hormone of the eyestalk acts directly on the tissues in restraining the onset of proecdysis, or whether, as Echalier (1954, 1955) and Gabe (1953) suggest, it acts in preventing the Y-organ from secreting a moult-promoting hormone which is essential for the processes of proecdysis and ecdysis to take place. Echalier has shown that extirpation of the Y-organ in *Carcinus* leads to a cessation of all the processes of proecdysis, so that a crab lacking this organ cannot enter proecdysis, nor continue further with it if it was part way through this stage when the operation was performed.

The Y-organs of 20 male *Carcinus* in the terminal anecdysis had a mean weight of 12·1 ± 1·1 mg, while 20 male *Carcinus* in the penultimate diecdysis had Y-organs with mean weight 13·0 ± 1·2 mg. When the weights, however, are expressed as a ratio of the body weight they become 10·9 ± 0·99 mg% and 21·1 ± 1·95 mg%. The difference appears to be significant at the 1% level. Relative to body size therefore the crabs in terminal anecdysis have smaller Y-organs than those slightly younger.

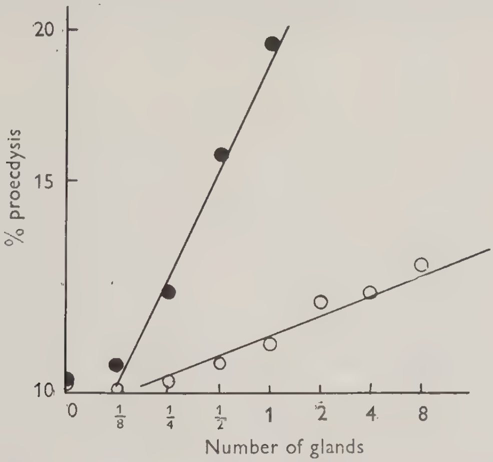

Fig. 2. Pooled results of a number of assays of the moult-promoting hormone of the Y-organ of *Carcinus* on *Leander*. The abscissa gives the number of glands supplying the extract which was injected into each individual; the ordinate gives the percentage of *Leander* which were found to be in proecdysis 5 days after the injections were made. Both scales are logarithmic. All the *Leander* were in diecdysis at the beginning of the assay. Donors in terminal anecdysis —open circles; donors in diecdysis—black circles.

An assay of the content of moult-promoting hormone in the Y-organ of *Carcinus* is illustrated graphically in Fig. 2. The Y-organs from crabs in diecdysis are significantly more potent ($P < 0.001$) in this respect than organs from crabs in the terminal anecdysis.

A single injection of an extract of Y-organ into *Carcinus* in terminal anecdysis resulted in a fourfold rise in blood-calcium level, lasting about 5 days (see Fig. 3). This may be taken as indicative of the beginning of proecdysis, albeit temporary. Accordingly I carried out a prolonged programme of injecting such *Carcinus* twice a week with Y-organ extracts. The extracts were prepared from younger crabs which were in the period of proecdysis, when *a priori* reasoning led me to believe that the titre of the moult-promoting hormone would be highest. Each crab received at each injection the extract of two

Y-organs. After 2 weeks on this regime 20 crabs all showed a heightened blood-calcium level, about four times that in the controls injected with an extract of leg nerve. One crab moulted after 37 days, three more in the next fortnight and one more on the 58th day. On the 60th day the remaining 12 crabs (three had died) were sacrificed, together with the 16 surviving control crabs. All of the latter were still in anecdysis, while all of the former were in late proecdysis. Repeated injections of Y-organ extract had therefore induced a further moult in crabs which had entered upon the terminal anecdysis.

Bilateral removal of the Y-organ from *Carcinus* in terminal anecdysis leads to no obvious effects. Bilateral eyestalk ablation following this operation led to a high mortality but sufficient survived to enable valid conclusions to be drawn.

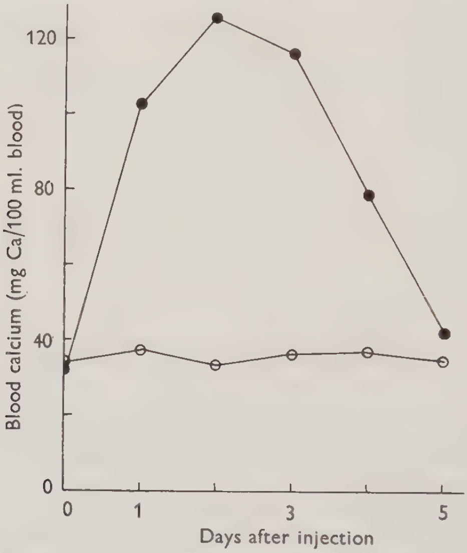

Fig. 3. The effect of injection of an extract of the Y-organ of *Carcinus* on the blood-calcium level of the same species during the terminal anecdysis. The controls (open circles) received an injection of extract of leg nerve.

Sixty days after the last stage of this double operation was completed not one of the 13 crabs which had survived so long had begun proecdysis; all were still in anecdysis. This may be contrasted with the results of simple eyestalk removal (see Table 1), when the crabs immediately began proecdysis, and had mostly moulted 60 days after the operation. We may conclude that the Y-organ is necessary for proecdysis to proceed, even in the absence of the moult inhibition afforded by the hormone of the eyestalk. It seems probable that the moult-inhibiting hormone of the X-organ-sinus gland complex of the eyestalk acts primarily in restraining the Y-organ from producing the moult-promoting hormone. It seems likely, however, that it has also a direct action on the tissues, for otherwise the slight titre of moult-promoting hormone produced by the inhibited Y-organ of the terminal anecdysis (see Fig. 2) would surely lead to a very slowly progressing proecdysis unless the tissues are actively inhibited to counterbalance its effects.

Maia squinado

Moult-inhibiting hormone

A comparative assay of sinus glands taken from the eyestalks of male *Maia* in the penultimate and the terminal anecdysis is illustrated in Fig. 4. It is evident from this that the titre of moult-inhibiting hormone is much lower—about eight times lower—during terminal anecdysis than during the penultimate anecdysis, despite the larger size of the older animals. Statistical analysis of the data gives the ratio between the titre per gland as 7·31, with 1% fiducial limits at 6·03 and 9·22. Expressed as titre per unit weight of tissue the ratio is 16·02 with 1% fiducial limits at 14·11 and 20·05. A similar assay using female *Maia* as donors gave comparable results.

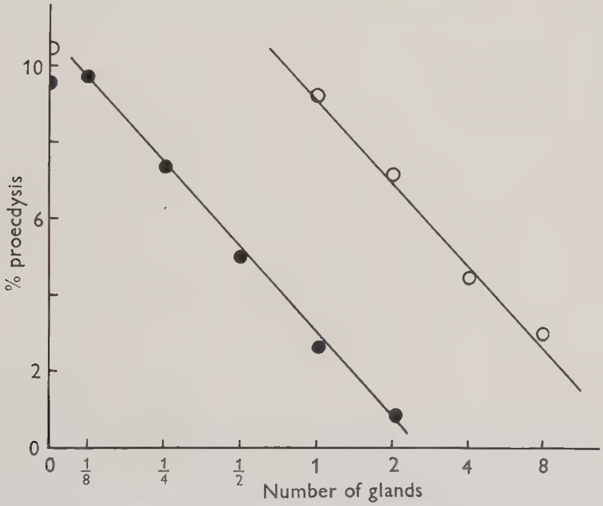

Fig. 4. Pooled results of a number of assays of the moult-inhibiting hormone of the sinus gland of *Maia* on *Leander*. The abscissa gives the number of glands supplying the extract which was injected into each individual; the ordinate gives the percentage of *Leander* which were in proecdysis 5 days after the injections were made. All the *Leander* were in diecdysis at the beginning of the assay. Donors in terminal anecdysis—open circles; donors in non-terminal anecdysis—black circles.

There can be little doubt that the failure to moult again once the terminal anecdysis has begun is not a result of excessive production of the moult-inhibiting hormone of the eyestalk—a situation quite contrary to that which we have found in *Carcinus*. In *M. squinado*, therefore, we must look elsewhere for the cause of the cessation of moulting.

The Y-organ of Gabe

During the penultimate anecdysis this organ is large and prominent, whereas during the terminal anecdysis it is much diminished in size so that it is often difficult to find upon dissection. During the penultimate anecdysis the Y-organs of 20 male *Maia* had a mean weight of 57·36 ± 7·32 mg, while those of 20 male *Maia* in the terminal anecdysis had a mean weight of 3·35 ± 0·92 mg. The difference is statistically very significant, with $P < 0{\cdot}001$.

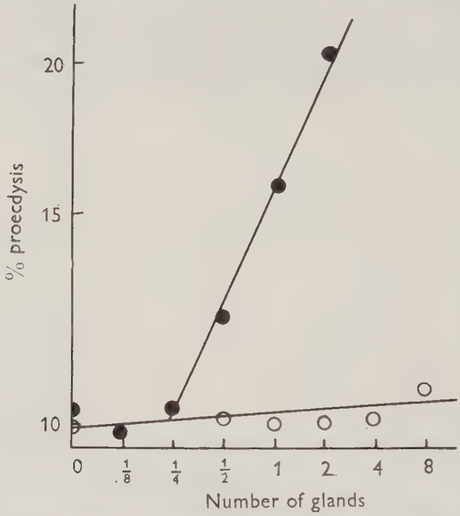

Fig. 5. Pooled results of a number of assays of the moult-promoting hormone of the Y-organ of *Maia* on *Leander*. The abscissa gives the number of glands supplying the extract which was injected into each individual; the ordinate gives the percentage of *Leander* which were found to be in proecdysis 5 days after the injections were made. Both scales are logarithmic. All the *Leander* were in diecdysis at the beginning of the assay. Donors in terminal anecdysis—open circles; donors in non-terminal anecdysis—black circles.

An assay of the moult-promoting hormone of the Y-organ of male *Maia* is illustrated graphically in Fig. 5. The regression line for the activity of the extracts prepared from the glands of crabs in the terminal anecdysis has a gradient which does not differ significantly from the horizontal, i.e. there is no noticeable effect of these extracts upon moulting. In contrast, the extracts prepared from glands of crabs in the penultimate anecdysis clearly have a pronounced effect in promoting the onset of proecdysis. The gradient of the regression line is 9·74 ± 1·33, which differs very significantly from zero ($P < 0{\cdot}01$).

A single injection of the extract prepared from ten Y-organs from *Maia* in the penultimate anecdysis, injected into each of ten crabs in the penultimate and ten in the terminal anecdysis, led in all of them to a transient threefold rise in the blood-calcium level, quite comparable to that found in *Carcinus* (Fig. 3).

This may be taken as a sign that the injection has led to a transient initiation of proecdysis in both groups of animals. A single injection of a similar extract prepared from the 25 Y-organs of *Maia* in the terminal anecdysis had no effect on the blood-calcium level in any of ten crabs.

We must conclude, then, that the absence of the moult-promoting hormone of the Y-organ during the terminal anecdysis in *Maia* is concerned in some way with the cessation of moulting. In this species the cessation of moulting is not a result of excessive production of moult-inhibiting hormone by the X-organ-sinus gland complex of the eyestalk. The cause of the functional degeneration of the Y-organ in the terminal anecdysis of *M. squinado* must be sought elsewhere than in the effects of the moult-inhibiting hormone of the eyestalk.

DISCUSSION

In *M. squinado* the last moult is that at which it attains sexual maturity, a state comparable with that found in insects. Once maturity is reached there is no further growth. Crustacea, however, suffer from one disability from which insects are free: copulation can only take place when the female has a soft integument and the male a hard one, that is, when the female is in the first stage of metecdysis and the male in some later stage of the moult cycle. In *Maia*, therefore, copulation is only possible when the female has just completed her final moult and the male has completed his some time previously. But the final moult in *Maia*, in Plymouth waters, takes place at a fixed time of year, in late July or August. It is thus impossible for the newly moulted male of that year to copulate with a newly moulted female: copulation must be between a male whose final moult took place one year or more previously and a newly moulted female. The heaps of *Maia*, which may be observed in July and August, are mixed heaps of moulting and copulating crabs (see Appendix). It is not until about 6 months after copulation that the eggs are finally fertilized, by sperm stored in the bursa copulatrix, and oviposited onto the pleiopods where they remain for about 9 months before hatching. A male, therefore, survives at least 12 months and a female at least 15 months after the final ecdysis. Nevertheless, once a *Maia* has entered upon the terminal anecdysis it may be considered as aged, for it is no longer capable of regenerating damaged tissue or limbs, or of repairing more than the most superficial injury. It is noteworthy in this respect that a *Maia* in terminal anecdysis is very much less ready than a younger crab to autotomize a damaged or captive limb. A mature *Maia* may be suspended by one leg out of water, even if the leg is damaged severely, without autotomizing the limb: it will die in this situation without casting the leg. A younger *Maia*, whose powers of regeneration are much greater, will cast the limb so treated and escape back to water with great rapidity. Echalier (1955) has reported that in the absence of the Y-organ, a crab cannot regenerate missing limbs, nor even produce the bud with which such regeneration normally begins.

The experiments reported in this paper suggest that in *Maia* the terminal anecdysis is brought about by the degeneration of the Y-organ. Correspondingly, no further ecdysis can begin, nor can regeneration of damaged parts. It seems likely that in the absence of predation, the death of a mature *Maia* is brought about by an accumulation of minor damage to the shell and tissues. It must also be remembered in this context that moulting in Crustacea often seems to serve as a mode of excretion (see Richards, 1951), since the exoskeleton stores waste material and especially nitrogen, which is discarded from the body on moulting. A crab in terminal anecdysis, therefore, is unable to use this method of excretion and may thus be constrained to accumulate toxic waste products in the tissues.

TABLE 2. INCREASE IN VOLUME OF *MAIA SQUINADO* AT MOULTING

Moult	Mean increase
Fourth before prepuberty	2·17
Third before prepuberty	2·15
Second before prepuberty	2·20
Before prepuberty	2·19
Prepuberty	2·26
First after prepuberty	2·20
Second after prepuberty	2·19
Last (puberty)	1·89

The increase in volume at each of the last eight moults is expressed as the ratio of the volume after moulting to that before it. Each ratio is the mean of at least fifteen observations and includes data of my own, supplemented by computations from the data of Teissier, 1934–55.

If the immediate controlling factor of terminal anecdysis in *Maia* is the degeneration of the Y-organ, we yet have no evidence as to the causation of this degeneration. All that can be said is that the inhibitory action of the X-organ-sinus gland complex is not the cause. The causative factor must be sought earlier in the life history. A clue to what is involved may be seen in the size increase which *Maia* undergoes at each moult. For most of the moults the relative increase in volume (in the male) is *ca.* 2·18 (see Table 2). At two moults only is the relative increase significantly different—the moult of prepuberty, when the gonads begin to increase in size and show the first signs of approaching maturity, and the moult of puberty three moults later, that is to say, the final ecdysis when the animal becomes sexually mature. At the prepuberal moult the relative increase in volume is *ca.* 2·26, greater, that is, than at other moults, while at the puberal moult the relative increase in volume is *ca.* 1·89, correspondingly less than at other moults. I have shown previously (Carlisle, 1955) that increase in volume at moulting is a function of the activity of the water balance hormone of the X-organ-sinus gland complex. It is possible that this is implicated in the control of the final ecdysis and may have some influence on the Y-organ, though such a concept must remain a mere speculation for the moment.

In *Carcinus maenas* we meet with rather a different situation. Here there can be little doubt that the main restraining influence preventing further moulting during the terminal anecdysis is over-production of the moult-inhibiting hormone of the X-organ-sinus gland complex. It seems likely that its prime action lies in restraining the Y-organ from producing the moult-promoting hormone, but nevertheless I believe that it must have some direct action on the tissues also; for otherwise the tissues would respond sooner or later, however slowly, to the small amounts of moult-promoting hormone which are produced by the incompletely inhibited Y-organ. What factor it is which leads the X-organ to produce the excess amounts of the moult-inhibiting hormone we have as yet no idea. But in *Carcinus* the terminal anecdysis only differs from earlier anecdyses as far as I have yet discovered in the higher titre of moult-inhibiting hormone. The control of the terminal anecdysis in this species seems to be merely an accentuation of the control of the earlier anecdyses and may well be gradually approached in these successive earlier anecdyses. *Carcinus* undergoes at least ten moults, eight diecdyses and two or three anecdyses between the moult of puberty and the terminal anecdysis (in the male, and probably also in the female). As I have repeatedly pointed out in previous papers (see Knowles & Carlisle, 1956) the control of anecdysis is different from that of diecdysis, and it is to this difference that we must look for origin of the terminal anecdysis in *Carcinus*.

One such difference between *Carcinus* in diecdysis and in anecdysis is in the level of secretion of the moult-inhibiting hormone of the X-organ-sinus gland complex. It will be obvious from a perusal of Fig. 1 that this difference is accentuated when we compare terminal anecdysis. During terminal anecdysis the eyestalk appears to be producing the moult-inhibiting factor faster than at any previous time in the life of the crab. There is thus in *Carcinus*, in contrast with *Maia*, a positive inhibition of the initiation of a further proecdysis once the animal has entered upon the terminal anecdysis. It is obvious, furthermore, that the Y-organ of *Carcinus* is not degenerate like that of *Maia* during the terminal anecdysis, and correspondingly a *Carcinus* in this stage is more capable of repairing minor damage to the shell. Once the inhibition to moulting is removed by surgical interference *Carcinus* enters upon a new proecdysis, stimulated to this, no doubt, by the moult-promoting hormone of the Y-organ. *Maia*, in contrast, lacks both the inhibiting factor and also the factor which might provoke renewed moulting.

It is evident that the type of control of the terminal anecdysis which is exemplified by *Carcinus* is more flexible than that found in *Maia*. Among the Portunidae, to which *Carcinus* belongs, there is great variation in the moulting cycle. At one extreme are to be found crabs like *Carcinus* and *Portunus depurator*, which have ten or a dozen moults after the moult of puberty, so that the terminal anecdysis does not coincide with sexual maturity, but rather begins several years later. At the opposite extreme are crabs such as *Callinectes* *sapidus* in which in the female the moult of puberty is the final moult, after which the animal is sexually mature and enters upon the terminal anecdysis, while in the male the moult of puberty appears to be several moults earlier—the copulatory appendages are present throughout the last few intermoult periods. Such variation between closely related species is hardly conceivable with the type of control of the terminal anecdysis found in *Maia*.

This investigation has served to reveal the existence of two distinct mechanisms whereby the terminal anecdysis, which is characteristic of the end of the life-span of most species of crabs, may be regulated. A mental review of the biology of crabs suggests that the mechanism found in *Maia* may well be characteristic of all the oxyrhynchan Brachyura, while that found in *Carcinus* may be characteristic of the brachyrhynchan Brachyura, or at least of the Portunidae. The mechanism found in *Maia* is reminiscent of the control of the final instar of insects and the organs concerned may be homologous (see Gabe, 1953). The dynamic equilibrium which seems to exist in *Carcinus*, on the other hand, appears to be unrelated to anything found in insects and to be a mechanism developed in the one group of crabs.

It must be emphasized that the hormonal influences which this investigation has shown to be effective in regulating the terminal anecdysis are only the immediate causes of this phenomenon. The mediate causes must be sought elsewhere as must the reason for the existence of the phenomenon. The problem of the terminal anecdysis, the ageing of crabs and the factors which cause natural senescence and death in these creatures is no more than touched, but in the hormonal factors concerned in inhibiting moulting we see an influence which is the last agent in the chain of events which brings about senescence.

SUMMARY

In many if not most species of crabs (but not quite all) there is a limit to growth, when no more moulting or ecdysis is possible in normal circumstances. This condition of permanent anecdysis is known as the terminal anecdysis. In the spider crab *Maia squinado*, the last moult is the moult of puberty, when the animal finally attains sexual maturity, and this moult has different biometrical characteristics from the others. In *Carcinus maenas*, in contrast, the moult of puberty takes place when the animal is quite small and may be succeeded by about ten further moults before the terminal anecdysis begins.

The immediate cause of the cessation of moulting in *Maia squinado* is shown to be the degeneration of the Y-organ, which secretes a moult-promoting hormone. In the absence of this gland and its secretion moulting can no longer continue. In *Carcinus* the Y-organ does not degenerate after the final ecdysis and the cause of the cessation of moulting is to be sought in the excessive production by the X-organ-sinus gland complex of the moult-inhibiting hormone. This effectively prevents moulting from proceeding.

Removal of the X-organ-sinus gland complex in *Carcinus* allows ecdysis to continue, so that giant crabs can be produced in the laboratory by this means, and at the same time the life-span may be increased. The operation has no such effect on *Maia*. In either species injection of Y-organ extracts produces transiently the first signs of an approaching moult, in the form of a heightened blood-calcium level. In *Carcinus* repeated injection led to eventual ecdysis. The presence of the Y-organ is shown to be necessary for removal of the X-organ-sinus gland complex to stimulate moulting in *Carcinus*.

Two separate methods of producing terminal anecdysis exist in crabs, both involving the hormones which normally regulate the moult cycle, but no reason is known for the existence of the phenomenon.

REFERENCES

BLISS, D. E., DURAND, J. B. & WELSH, J. H., 1954. Neurosecretory systems in decapod Crustacea. *Z. Zellforsch.*, Bd. 39, pp. 520–36.

BROWN, F. A. & CUNNINGHAM, O., 1939. Influence of the sinus-gland of crustaceans on normal viability and ecdysis. *Biol. Bull., Woods Hole*, Vol. 77, pp. 104–14.

CARLISLE, D. B., 1953. Moulting hormones in *Leander* (Crustacea Decapoda). *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 289–96.

—— 1954. On the hormonal inhibition of moulting in decapod Crustacea. I. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 61–3.

—— 1955. On the hormonal control of water balance in *Carcinus*. *Pubbl. Staz. zool. Napoli*, Vol. 27, pp. 227–31.

CARLISLE, D. B. & DOHRN, P. F. R., 1953. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). II. Experimental evidence for a growth- and moult-accelerating factor obtainable from eyestalks. *Pubbl. Staz. zool. Napoli*, Vol. 24, pp. 69–83.

CORNUBERT, G., DÉMEUSY, N. & VEILLET, A., 1952. Effets de l'ablation des pédoncules oculaires sur le développement des caractères sexuels externes des Décapodes Brachyoures *Carcinus maenas* Pennant et *Pachygrapsus marmoratus* Fabricius. *C.R. Acad. Sci., Paris*, T. 234, pp. 1405–7.

DÉMEUSY, N., 1953. Comparaison biologique de deux populations de *Carcinus maenas* Pennant: mue de puberté. *C. R. Acad. Sci., Paris*, T. 236, pp. 1203–5.

DÉMEUSY, N. & VEILLET, A., 1953. Sur l'existence de deux populations de *Carcinus maenas* Pennant et sur les caractères morphologiques qui les distinguent. *C.R. Acad. Sci., Paris*, T. 236, pp. 1088–90.

ECHALIER, G., 1954. Recherches expérimentales sur le rôle de 'l'organe Y' dans la mue de *Carcinus moenas* (L.), Crustacé décapode. *C.R. Acad. Sci., Paris*, T. 238, pp. 523–5.

—— 1955. Rôle de l'organe Y dans le déterminisme de la mue de *Carcinides* (*Carcinus*) *moenas* L. (Crustacés décapodes). Expériences d'implantation. *C.R. Acad. Sci., Paris*, T. 240, pp. 1581–3.

FINNEY, D. J., 1952. *Statistical Method in Biological Assay*. London, 661 pp.

GABE, M., 1953. Sur l'existence, chez quelques Crustacés Malacostracés, d'un organe comparable à la glande de la mue des Insectes. *C.R. Acad. Sci., Paris*, T. 237, pp. 1111–13.

HIATT, R. W., 1948. The biology of the lined shore crab, *Pachygrapsus crassipes* Randall. *Pacif. Sci.*, Vol. 2, pp. 135–213.

KNOWLES, F. G. W. & CARLISLE, D. B., 1956. Endocrine control in the Crustacea. *Biol. Rev.*, Vol. 31, pp. 396–473.

PASSANO, L. M., 1953. Neurosecretory control of moulting in crabs by the X organ-sinus gland complex. *Physiol. comp.*, Vol. 3, pp. 155–89.

RICHARDS, A. G., 1951. *The Integument of Arthropods.* Minneapolis, 411 pp.

TEISSIER, G., 1934. Différences sexuelles dans la croissance des appendices de *Maia squinado* (L.). *C. R. Soc. Biol., Paris*, T. 117, pp. 668–71.

—— 1935. Croissance des variants sexuelles chez *Maia squinado* (L.). *Trav. Sta. biol. Roscoff*, Fasc. 13, pp. 93–130.

—— 1937. Allométrie de taille et variabilité. *C.R. Soc. Biol., Paris*, T. 124, pp. 1071–3.

—— 1955*a*. Sur la détermination de l'axe d'un nuage rectiligne de points. *Biometrica*, Vol. 11, pp. 344–56.

—— 1955*b*. Allométrie de taille et variabilité chez *Maia squinado*. *Arch. Zool. exp. gén.*, T. 92, pp. 221–64.

APPENDIX

A number of reports have appeared in the lay press and in fishing journals of the occurrence of heaps of spider crabs (*Maia squinado*) at various points around the coast. One such account is given of large heaps 'about two feet high and three feet in diameter' on the coast of Jersey during the autumn of 1952, by Le Sueur (1953)[1]. He interpreted these heaps, which were observed by fishermen and by the president (an amateur zoologist) of the Société jersiaise, as a means of protection against *Octopus* which will successfully attack crabs larger than themselves. While congregation for mutual defence is certainly a major part of the explanation of such a heap it is by no means all. Some observations which I was able to make during July to September 1956, on a heap of *Maia* at Mothecombe, South Devon (50° 18·3′ N., 3° 57·9′ W.) have enabled me to supplement this explanation.

Like those in Jersey, the heap at Mothecombe was about 1 m in diameter, or rather more, and 0·5 m or more high. It consisted of about 60 *Maia*. When first observed on 12 July the top of the heap was about 1 m below low tide level, and the depth of water at that point was, therefore about 1·5 m. From the tide tables it is apparent that there was never less than 90 cm of water above the top of the heap, at any stage of the tide or phase of the moon, for the tide on 12 July was a spring tide and low water prediction was 0·5 ft (15 cm) below chart datum (the mean level of low water spring tides throughout the year). The crabs in the heap remained almost completely stationary even when sand was thrown in a cloud over them. The animals of the bottom layer of the heap were clinging to an isolated rock about 1·2 by 0·9 m, which was completely surrounded by sand. This rock stood about 20 cm above the surrounding sand and lay about 60 cm from the main reef which ran close by. A moderately heavy growth of *Laminaria*, chiefly *L. saccharina*, was attached to the rock and fronds rose through the heap of crabs, floating about above their backs, providing a partial cover. About twenty of the crabs were full-grown males, in terminal anecdysis; the rest were about equal numbers of smaller, immature males and females, of a size which indicated that they were in the penultimate instar. During the course of the next month the heap was swollen by the addition of about a further 20 crabs, making 80 in all. On 25 July a newly cast shell lay beside the heap and I located a soft male in the centre of the heap. During the next three weeks one or two crabs moulted every day, and always the soft crabs were to be found in the centre of the heap, not on the surface. As time wore on it became apparent that each female as soon as she moulted entered into copulation with one of the old, hard males. So that by mid-August six or eight pairs could be observed in copula at one time. By mid-September the heap was beginning to disperse, but had not completely done so when equinoctial gales rendered the sea too opaque to continue observations.

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[1] *Bull. Soc. jersiaise*, Vol. 16, pp. 37–8.

During the summer of 1956 *Octopus* were too rare around the Devon coasts to be a major predator of *Maia*, and so far as I am aware no other common animal of the English fauna is capable of attacking a hard *Maia*. A soft crab, however, is vulnerable to attack from a great many predators, for not only is its shell soft, but its muscular system is inefficient until the skeleton is hardened. Most brachyrhynchan crabs before and after moulting hide away in crannies amongst the rocks, or buried in the sand. *Maia* from its very shape is incapable of doing either. With most species of crabs, which are carnivorous, the predator which is most likely to attack a soft crab is another crab. *Maia*, however, is not a carnivore and, therefore, will not attack another of the species, even when soft. It is, therefore, feasible for them to congregate together for mutual protection when moulting, an unthinkable happening in a carnivorous species of crab. It seems to me reasonable to suppose that this is in fact the primary purpose of the heaps, to which is inevitably added the secondary purpose of mating. Copulation could equally well take place between pairs of crabs in isolation, as happens in other species of crabs, but if there is communal moulting then it will take place where moulting happens. It seems not improbable that the seasonal moulting of *Maia*, which only takes place in July and August and not at other times of the year, has arisen because of this method of mutual protection that this species has developed.

In support of my contention that *Maia* is not a carnivorous species of crab I will mention observations I have made at Mothecombe on the feeding of this species. In addition to the heap of crabs numerous isolated individuals were observed, mainly animals in terminal anecdysis. Many of these, when observed closely, were found to be feeding by browsing on tufted growths, both animal and plant. Such plants as *Enteromorpha*, *Corallina*, *Heterosiphonia* and *Griffithsia*, and animals such as tufted Bryozoa and hydroids appeared to be the main foods of *Maia*. The crabs were often to be seen browsing directly on the ends of the longer tufts, while the shorter organisms were detached from the substratum or broken by the chelae and thence conveyed to the mouth. It is an undoubted fact that *Maia* are often taken in crab pots, enticed there by the bait, but in the wild it seems unlikely that fresh or decaying flesh forms any major part of the diet. The enlarged chelae of the mature male seem to serve especially in fighting, or rather in warding off other males, while coupling with a female. Any fights which may be observed are slow moving affairs and I have never seen any sign of damage to an animal resulting from this cause.

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SOME QUANTITATIVE ASPECTS OF FEEDING IN SABELLID AND SERPULID FAN WORMS

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INTRODUCTION

It is apparent, from Jørgensen's recent review (1955) of quantitative aspects of filter feeding in invertebrates, that virtually nothing is known of the rate of filtering in polychaete suspension feeders, of which the sabellid and serpulid fan worms are perhaps the most important.

There seems to be little variation in the filter-feeding mechanism in different sabellid and serpulid polychaetes. The most detailed account of the feeding mechanism of these worms is that of *Sabella* published by Nicol (1930) with a résumé of earlier work on sabellids and serpulids. Some information on feeding and the anatomy of the crown in other genera may be found in the works of Soulier, 1891 (*Serpula*, *Hydroides*, *Protula*, *Branchiomma*, *Spirographis* and *Myxicola*), Johansson, 1927 (*Serpula* and *Pomatoceros*) and Thomas, 1940 (*Pomatoceros*). That the crown arises as a paired structure from the prostomium was shown by Wilson (1936) in *Branchiomma*. In adults, the crown may retain a clearly divided form, as in many serpulids such as *Pomatoceros*, or form an almost continuous single cone as in *Myxicola* or *Salmacina*. It is not, however, the purpose of the present paper to describe these variations in morphology, but to present some quantitative data on the filtering process. The species investigated were those which could be obtained in sufficient quantity at Plymouth. The results of experiments on the sabellids, *Myxicola infundibulum* (Rénier) and *Sabella pavonina* Savigny, and on the serpulids, *Pomatoceros triqueter* (L.), *Hydroides norvegica* (Gunnerus), *Spirorbis borealis* Daudin, and *Salmacina dysteri* (Huxley) are presented here.

This work was done during the summer of 1956 at Plymouth, and I am grateful to the Director and Staff of the Laboratory for all the various facilities that made the work possible. I wish to thank especially Dr Dorothy Ballantine for supplying the algal cultures used, Mr R. F. H. Freeman, of Queen Mary College, for kindly lending me his absorptiometer, and Prof. G. P. Wells, F.R.S., for helpful criticism of the typescript of this paper, and discussion of the results.

METHODS

The total volume of water strained, not the actual quantity of particles ingested, has been measured. Suspensions of 'Aquadag', a colloidal graphite suspension in water supplied by Acheson Colloids Ltd., London, was used. The method depends on the assumption that all the particles in the water filtered are removed from suspension, and those remaining are evenly distributed. The removal of particles from a closed system is exponential and as the filtering rate in fan worms was found not to vary with density, the volume and initial density of suspension could be adjusted to any convenient value. Both Jørgensen (1949), working with *Mytilus*, and Ballantine & Morton (1956), working with another lamellibranch, *Lasaea*, have found graphite suspensions to give variable results. Nevertheless, all the worms investigated fed and behaved normally in 'Aquadag' suspensions, and the values for filtering rates obtained here were consistent for each species. The suspensions were made up in the following way. A knife-point of the graphite paste was shaken vigorously in a test-tube with distilled water for 1–2 min, and then poured into a small beaker. After 5 min the resulting suspension was carefully decanted into a bottle, made up to 50 ml. with distilled water and allowed to stand a further 5 min. Such freshly made-up suspensions were used for each experiment and were found to give even suspensions consisting of particles almost entirely 1–2 μ in size, with a few larger particles up to 4–5 μ. In distilled water such dilutions are stable for at least some days. For the experiments, 1–2 ml. of this stock suspension was added to about 250 ml. of sea water. After 4 h all the particles were less than 10 μ and most 1–2 μ across. After 12 h, aggregates up to 50–60 μ had appeared; 18 h afterwards, 100 μ aggregates had formed, and after 24 h the aggregates rapidly sedimented. The optical density decreases during this aggregation process, but not materially during the first 4 h after preparing the sea-water suspension. The density of the suspension and the number of animals used in each experiment was therefore adjusted to give an easily measurable value in 2–3 h. The rate of filtering was calculated from the formula used by Jørgensen (1943) from measurements of the decrease in optical density of the suspension by means of an absorptiometer. Jørgensen (1949) found a change in feeding rate during the experiments using colloidal graphite with *Mytilus*. This may have been due to the suspensions being initially uneven, rather than to more rapid aggregation of small particles. Only by following the shaking and decanting process described here was it found possible to obtain even and stable suspensions. In some test experiments with *Myxicola*, estimation of filtering rate using suspensions in sea water, 12 and 18 h old, did not give values significantly different from those obtained with freshly made suspensions. While much of the graphite was ingested by the worms in all experiments, some was rejected, probably owing to the rather dense suspensions used. As all the particles caught on the pinnules become entangled in mucus, however, the fall in optical density of the suspension gives a measure of the volume strained through the crown. Probably under natural conditions, where the density of small particles in suspension is much less, the majority of suitable particles would be ingested, so that the filtering rate becomes equivalent to the feeding rate.

Animals were acclimatized for some days to the experimental vessels kept under circulation before measurement of filtering rate. Care was taken to see that the animals were filtering and behaving normally before each experiment. Most species, especially *Sabella*, were found to become much less sensitive to shock if the vessels containing the animals were handled repeatedly for a day or so before the experiment. This was done so that the supernatant water could be sampled for measurement of optical density without causing retraction of the worm and interruption of feeding. Momentary, and apparently spontaneous, retractions take place, but the recovery and resumption of filtering is more prompt than after a shock reaction. *Hydroides* was found the most sensitive to experimental conditions. The *Sabella* (in their own tubes), were arranged individually, in small specimen tubes cemented to the bottom of the vessel, so that the crowns could be expanded and twirled without obstruction. *Myxicola* was allowed to secrete new tubes in the bottom of the vessel before being used in an experiment. Vessels with a capacity of 750 ml. were used for these large sabellids, with 1–3 worms in each. For the experiments on *Pomatoceros*, animals were selected with their tubes cemented to pebbles of convenient size, and these scraped clean of other filter-feeding animals before being acclimatized to vessels of 250–300 ml. capacity. *Hydroides*, growing on *Pecten* and *Buccinum* shells, were placed in 100 ml. vessels; pieces of *Salmacina* colony, and *Spirorbis* growing on *Fucus*, were placed in 10 ml. vessels for measurement of their filtering rates.

The worms used in the experiments were dried on filter paper and weighed; the larger species individually. *Spirorbis* and *Salmacina* were weighed in batches. Ten worms were placed on a cover-slip, the crowns dissected off, and surplus moisture drained off with filter paper; the whole process being performed under a binocular microscope. After weighing, the crowns were removed, and the cover-slip weighed again; the difference was taken as the weight of the crowns. Weighing was performed on an aperiodic microchemical balance with a sensitivity of 0·01 mg. In serpulids, the operculum and its stalk were dissected off, and, while included in the total body weight, are excluded from the weight of the crown. The extreme values for the weights of *Salmacina* and *Spirorbis* (Table 1, column 4) represent the means of ten of the largest and ten of the smallest selected from those used in the experiments.

TABLE 1. RESULTS OF EXPERIMENTS ON FILTERING RATES USING COLLOIDAL GRAPHITE SUSPENSIONS

(The figures in parentheses represent the number of animals or experiments from which each calculation is derived)

	1 Total number of animals used	2 Total number of experiments	3 Arithmetic mean of total (fresh) weight (g)	4 Extreme total (fresh) weights (g)	5 Temperature range of experiments (° C)	6 Arithmetic mean filtering rate/animal (l./h)	7 Standard deviation of mean filtering rate/animal
Myxicola infundibulum	137	45	2·695 (65)	1·410–4·360	17–20	0·28600 (120)	0·06323
Sabella pavonina	150	50	0·187 (45)	0·043–0·236	18–20	0·07300 (150)	0·04266
Pomatoceros triqueter	42	45	0·0185 (42)	0·0117–0·0273	16–17	0·02700 (42)	0·01557
Hydroides norvegica	41	44	0·0123 (41)	0·0081–0·0235	16–17	0·01116 (44)	0·04908
Spirorbis borealis	2500	99	0·00024 (75)	0·000089–0·00052	17–19	0·00023 (2475)	0·00016
Salmacina dysteri	340	50	0·00014 (56)	0·00012–0·00015	17–18	0·00029 (340)	0·00012

	8 Arithmetic mean filtering rate/unit total (fresh) weight (l./h/g)	9 Mean crown weight (g)	10 Standard deviation of crown weight	11 Arithmetic mean filtering rate/unit crown weight (l./h/g)	12 Ratio of total weight to weight of crown	13 Standard deviation of ratio
Myxicola infundibulum	0·10 (120)	0·323 (24)	0·1118	0·842 (48)	7·99 (21)	0·905
Sabella pavonina	0·39 (150)	0·0195 (47)	0·00486	3·620 (48)	9·48 (48)	5·400
Pomatoceros triqueter	1·40 (42)	0·004 (36)	0·000415	5·400 (42)	4·62 (42)	0·832
Hydroides norvegica	0·90 (41)	0·00256 (41)	0·000806	2·300 (41)	4·81 (41)	0·976
Spirorbis borealis	0·95 (75)	0·00008 (50)	0·000003	5·320 (75)	2·99 (50)	0·279
Salmacina dysteri	2·09 (56)	0·00005 (50)	0·0000038	5·860 (56)	2·80 (50)	0·235

DISCUSSION OF THE RESULTS

The results of all the experiments using colloidal graphite suspensions are summarized in Table 1. The detailed results need not be given here; measurements and calculations of general interest are included in the table, and the consistency of the values obtained may be judged from the standard deviations given, bearing in mind the random selection of the animals and their size range. About fifty experiments were performed on each species.

It is not surprising that the largest species, *Myxicola infundibulum*, should have the highest individual filtering rate (286 ml./h), which is over 1000 times that of *Salmacina* and *Spirorbis* (0·23–0·29 ml./h). On the other hand, the size range within the group is very great; the weight of *Myxicola* is over 25,000 times that of *Salmacina*. Thus the largest species have lower filtering rates per unit of weight than the smallest. This is in accordance with the general dictum that larger animals have lower metabolic rates than smaller ones within a phylogenetic series. The respiration rates of the smaller serpulids have not been measured, but Wells (1952) found the respiration rate of *Myxicola* to be 0·035 ml. O_2/g fresh weight/h, and *Sabella* about 0·05 ml. O_2/g/h, a similar value for *Sabella* having been found also by Ewer & Fox (1940). It is of interest to note also that, as far as is known, the larger species with lower filtering rates have a longer life span than the smaller. *Spirorbis borealis* lives for a few months only, *Pomatoceros* for at least two or three years (Robertson & Pantin, 1938), and a single *Sabella* has been recorded as living in the Plymouth Aquarium for at least ten years (Wilson, 1949).

If the filtering rate is expressed as a function of the crown weight alone, it is found that the rates are not significantly different in any of the species studied. The higher filtering rates per unit total weight in the smaller species are therefore not attained by crowns which for some structural reason are more efficient—that is, capable of straining a larger quantity of water per unit weight and time—than those of larger species, but rather of having relatively larger crowns. In *Spirorbis*, the crown represents a third of the total weight, in *Hydroides* about a fifth, in *Sabella* only a ninth. Conversely, the greater apparent complexity of the crown in *Myxicola* must be related to other factors. While both *Myxicola* and *Sabella* are found at the mouths of estuaries in mud banks, *Sabella* builds a tube projecting well above the surface, so that when the crown is expanded it is held away from the bottom. The slimy tubes of *Myxicola* are completely buried, so that the expanded crown only just projects from the surface. The inter-filamentary membranes probably prevent the crown from becoming clogged, the current bearing the finer suspended particles on which the worms feed entering from the rim. As Wells (1952) noticed, the pinnules are somewhat rotated so that the ventro-lateral cilia have a stronger longitudinal, as opposed to transverse, component with reference to the axis of the filament. The high standard deviation of the ratio of crown to total body weight (Table 1, column 13) in *Sabella* is due to some of the animals having partially regenerated crowns.

With the larger species it was not found practicable to measure the filtering rate with algal cultures owing to the very large volume which would be required. A few experiments using *Phaeodactylum tricornutum* Bohlin with *Myxicola*, and a number using cultures of *Phaeodactylum*, *Isochrysis galbana* Parke, and *Chromulina pusilla* Butcher, were made with *Spirorbis*. These three species of algae were selected, not only because of their availability at the Plymouth Laboratory, but because of their size differences: *Phaeodactylum* cells are 20–40 μ; *Isochrysis*, 5–6 μ; *Chromulina*, 1–2 μ in length. All the fan worms avoided dense suspensions of *Phaeodactylum* and *Isochrysis*, and even when much diluted the filtering rates were very small compared with those obtained with colloidal graphite. *Myxicola* and *Spirorbis* both removed *Phaeodactylum* from suspension at less than a tenth of the rate recorded with colloidal graphite. The other fan worms would not keep their crowns expanded in dense suspensions of these algae. This is certainly not merely a matter of size. *Myxicola* in 18 h old 'Aquadag' suspensions filtered at the same rate as in freshly made suspensions in which the majority of the particles were less than 2 μ, and which had a greater density than the *Phaeodactylum* suspensions used. *Chromulina* are closely similar to such fresh graphite particles in size, but dense suspensions of this alga produced repeated retractions of the crown in *Spirorbis*. The *Spirorbis* would remain with the crown expanded in more dilute cultures, but the filtering rate was negligible. One can only conclude that these algae can escape from the pinnules which are thus only capable of filtering inert particles of detritus from suspension. This is perhaps one explanation of the abundance of fan worms at the mouths of estuaries where there is much fine detritus in suspension.

During the present work a few experiments were made on *Chaetopterus*, using 'Aquadag' suspensions. Using seventeen different animals in their own tubes in groups of 1–3, allowing 1·5 l. of suspension for each group, and measuring the filtering rate over a 2 h period, it was found that at 16° C the mean filtering rate was 0·3 l./h/animal (standard deviation 0·183 l./h). The mean fresh weight of these animals was 6·0 g, giving a value of 0·05 l./h/g. Jørgensen (1955) calculated the filtering rate as 0·96 l./h from data given by Wells (Wells & Dales, 1951), but this was based on a calculation of the flow through the tube of one worm on a single occasion. The highest value obtained here over the 2 h period was 0·75 l./h, in a vessel containing a single animal. It is known from the work of MacGinitie (1939) and Wells (Wells & Dales, 1951) that *Chaetopterus* may both irrigate the burrow without feeding and feed without secreting a complete mucus bag such as might be expected to retain all the graphite particles in the water strained. It is also known that such activities are discontinuous. The value for the 'filtering rate' given here may, therefore, bear little relation either to the feeding rate or the total volume of water passed, and is not comparable with the values for fan worms which filtered more or less continuously during the course of an experiment. It is impossible to observe the activity of the animals in their own tubes as these are opaque.

The values obtained for the fan worms (Table 1) are all low compared with known values for other invertebrates. For example, from the information given by Jørgensen (1955), derived from various sources, *Ostrea virginica* filters at the rate of about 0·5 l./h/g, *Mytilus edulis* at 1·6 l./h/g and *Ciona intestinalis* at 0·23 l./h/g, at comparable temperatures. *Ciona* has much the same fresh weight as *Myxicola*, which strains at less than half this rate (0·1 l./h/g). Clearly the fan worms are less efficient than other suspension-feeding invertebrates, both in the volume of water they are capable of straining, and in the kind of particles which can be retained. While some sorting according to size occurs in *Sabella* (Nicol, 1930) and in other fan worms, this is crude compared with sorting mechanisms in some other suspension-feeding invertebrates.

SUMMARY

The rate of filtering has been examined in a series of sabellids and serpulids of widely different size, using suspensions of colloidal graphite and algal cultures. The filtering rates have been expressed as individual rates, as the volume strained per unit fresh weight, and per unit weight of the crown. It may be concluded that the smaller fan worms filter at a relatively higher rate than the larger. This is achieved partly by the relatively larger crown. Free swimming algae escape through the crown; only inert particles down to 1–2 μ are retained, implying that fan worms depend on suspended detritus alone. The filtering rates are briefly compared with those of *Chaetopterus* and some other filter-feeding invertebrates.

REFERENCES

Ballantine, D. & Morton, J. E., 1956. Filtering, feeding, and digestion in the lamellibranch *Lasaea rubra*. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 241–74.

Ewer, R. F. & Fox, H. M., 1940. On the function of chlorocruorin. *Proc. roy. Soc.* B, Vol. 129, pp. 137–53.

Johansson, K. E., 1927. Beiträge zur Kenntnis der Polychaeten-Familien Hermellidae, Sabellidae und Serpulidae. *Zool. Bidr. Uppsala*, Bd. 11, pp. 1–183.

Jørgensen, C. B., 1943. On the water transport through the gills of bivalves. *Acta physiol. scand.*, Vol. 5, pp. 297–304.

—— 1949. The rate of feeding by *Mytilus* in different kinds of suspension. *J. mar. biol. Ass. U.K.*, Vol. 28, pp. 333–44.

—— 1955. Quantitative aspects of filter feeding in invertebrates. *Biol. Rev.*, Vol. 30, pp. 391–454.

MacGinitie, G. E., 1939. The method of feeding of *Chaetopterus*. *Biol. Bull., Woods Hole*, Vol. 77, pp. 115–18.

NICOL, E. A. T., 1930. The feeding mechanism, formation of the tube, and physiology of digestion in *Sabella pavonina*. *Trans. roy. Soc. Edinb.*, Vol. 56, pp. 537–98.

ROBERTSON, J. D. & PANTIN, C. F. A., 1938. Tube formation in *Pomatoceros triqueter* (L.). *Nature, Lond.*, Vol. 141, pp. 648–9.

SOULIER, A., 1891. Études sur quelques points de l'anatomie des Annélides tubicoles de la région de Cette. *Trav. Inst. Zool. Univ. Montpellier*, T. 2, pp. 1–310.

THOMAS, J. G., 1940. *Pomatoceros, Sabella* and *Amphitrite*. *L.M.B.C. Memoirs*, No. 33, 88 pp. Liverpool: University Press.

WELLS, G. P., 1952. The respiratory significance of the crown in the polychaete worms *Sabella* and *Myxicola*. *Proc. roy. Soc.* B, Vol. 140, pp. 70–82.

WELLS, G. P. & DALES, R. P., 1951. Spontaneous activity patterns in animal behaviour: the irrigation of the burrow in the polychaetes *Chaetopterus variopedatus* Renier and *Nereis diversicolor* O. F. Müller. *J. mar. biol. Ass. U.K.*, Vol. 29, pp. 661–80.

WILSON, D. P., 1936. The development of the sabellid *Branchiomma vesciculosum*. *Quart. J. micr. Sci.*, Vol. 78, pp. 543–603.

—— 1949. Notes from the Plymouth Aquarium. *J. mar. biol. Ass. U.K.*, Vol. 28, pp. 345–51.

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PHOSPHORUS AND SILICON IN SEA WATER OFF PLYMOUTH DURING 1955

By F. A. J. Armstrong

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(Text-figs. 1–4)

Analyses of sea water collected during 1955 at the International Hydrographic Station E1 (lat. 50° N., long. 4° 22′ W.) are here presented in the same form as in previous reports (Armstrong, 1954, 1955). The methods of collection and analysis were substantially unchanged.

Temperature and salinity

The minimum surface temperature recorded was 7·7° C on 15 March, the maximum 18·53° C on 13 July. A quite surprising fall in surface temperature took place between 13 July and 11 August, during a spell of unusually warm weather. Temperatures and salinities on these dates are shown in Table 1. During July and August air temperatures recorded at Mount Batten meteorological station, Plymouth, were as follows:

July	Mean max. 22·0° C	Mean min. 13·1° C	Mean 17·6° C
Aug.	Mean max. 22·4° C	Mean min. 14·7° C	Mean 18·6° C

Winds were mainly light north-east and east.

The vertical distribution of temperature at E1 is shown in Fig. 1. At the beginning of the year temperatures were, as is usual, uniform from top to bottom of the water column, some surface warming becoming just apparent in April. By 9 May there was a slight temperature gradient in the column, but no marked thermocline; one was, however, well established at about 30 m on 13 June, and persisted, with some fluctuation in depth, until September. By 18 October the water column was again isothermal.

The highest salinities were recorded on 15 March (mean 35·27‰). During summer the values were low (34·89‰ on 11 August), rising in the autumn, though November (35·04‰) was significantly lower than October (35·17‰) or December (35·13‰). The change in salinity below 25 m between 13 July and 11 August, shown in Table 1, is probably significant of a change in water mass.

Phosphate

The winter maximum was notably high, the integral mean values of 0·59 and 0·58 μg atom P/l. for February and March being the highest recorded since 1929. In an attempt to determine the extent and, if possible, the source

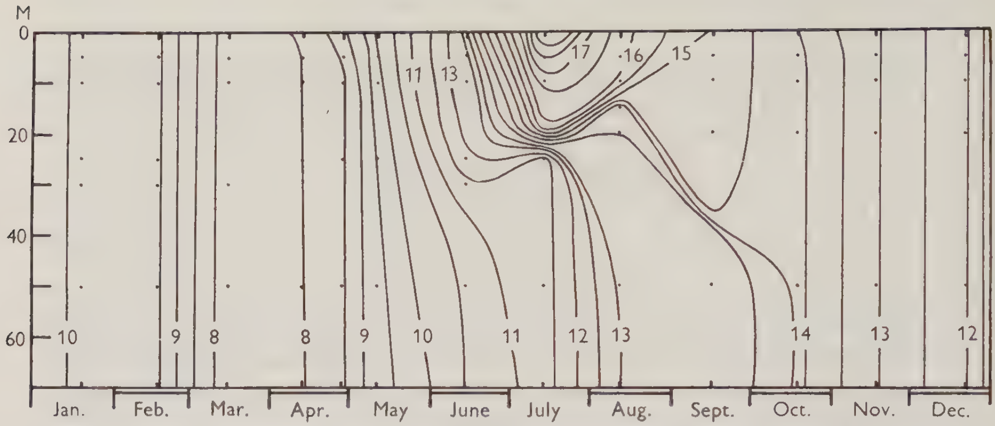

Fig. 1. Vertical temperature distribution at International Hydrographic Station E 1, 1955. Contour lines at 0·5° C intervals.

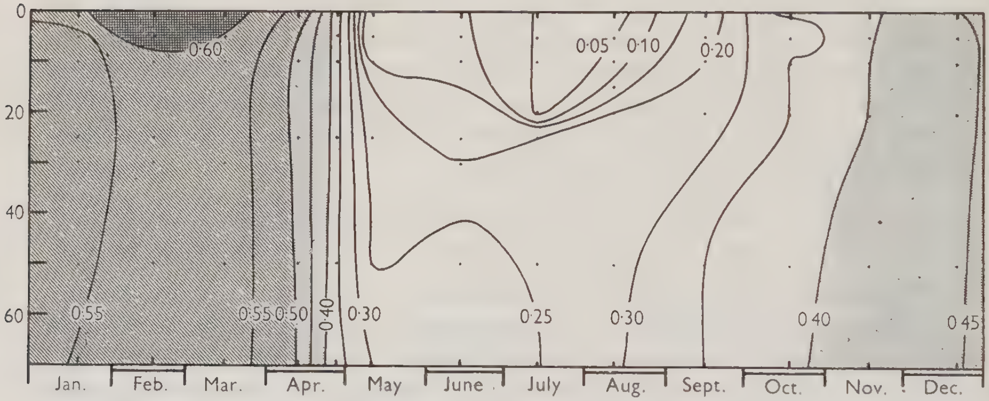

Fig. 2. Vertical distribution of phosphate at International Hydrographic Station E 1, 1955. Contour lines at 0·05 μg atom P/l. intervals.

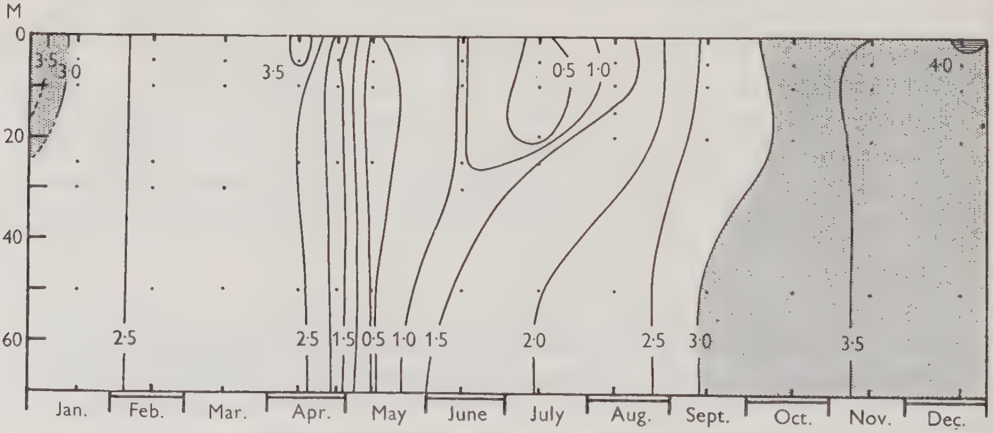

Fig. 3. Vertical distribution of silicate at International Hydrographic Station E 1, 1955. Contour lines at 0·5 μg atom Si/l. intervals

TABLE 1. TEMPERATURES AND SALINITIES AT INTERNATIONAL HYDROGRAPHIC STATION E1, 13 JULY AND 13 AUGUST 1955

Depth (m)	Temperature (° C) 13 July	Temperature (° C) 11 Aug.	Salinity (‰) 13 July	Salinity (‰) 11 Aug.
0	18·53	16·20	34·97	34·98
5	17·55	16·15	34·92	34·91
10	16·77	16·04	34·93	34·90
15	—	13·75	—	34·90
20	15·27	13·49	34·93	34·91
25	11·36	—	34·93	—
50	11·30	13·07	34·96	34·85
72	11·22	13·07	34·93	34·89

TABLE 2. INTEGRAL MEAN CONCENTRATIONS IN WATER COLUMN AT STATION E1

Date	Phosphate-P (μg atom P/l.)	'Total-P' (μg atom P/l.)	Silicate (μg atom Si/l.)
18. i. 55	0·54	0·61	2·12
16. ii. 55	0·59	0·70	2·82
15. iii. 55	0·58	0·71	2·65
12. iv. 55	0·49	0·62	2·72
27. iv. 55	0·36	—	1·77
9. v. 55	0·22	0·49	0·39
13. vi. 55	0·22	0·31	1·33
13. vii. 55	0·17	0·35	1·40
11. viii. 55	0·19	0·39	1·87
15. ix. 55	0·25	0·44	2·94
18. x. 55	0·32	0·47	3·11
17. xi. 55	0·36	0·54	3·68
21. xii. 55	0·38	0·54	3·26

TABLE 3. POSITIONS OF STATIONS AND PHOSPHATE CONCENTRATIONS AT 10 m DEPTH, WESTERN APPROACHES, 14–21 MARCH 1955

Date	Station no.	N. Lat.	W. Long	Phosphate at 10 m (μg atom P/l.)
14. iii. 55	1 (E2)	49° 27′	4° 42′	0·58
	2 (E3)	48° 34′	5° 13′	0·15
15. iii. 55	3	47° 46′	6° 05′	0·58
	4	47° 20′	6° 28′	0·56
16. iii. 55	5	46° 30′	8° 00′	0·56
17. iii. 55	6	47° 14′	7° 55′	0·55
	7	47° 50′	7° 40′	0·63
18. iii. 55	8	48° 18′	7° 30′	0·60
	9	49° 00′	9° 00′	0·60
	10	49° 47′	10° 15′	0·63
19. iii. 55	11	50° 35′	11° 28′	0·72
	12	50° 34′	11° 10′	0·76
	12A	50° 32′	10° 56′	0·72
	12B	50° 32′	10° 50′	0·75
	13	50° 19′	10° 20′	0·64
20. iii. 55	14	50° 07′	8° 43′	0·63
	15	49° 50′	8° 00′	0·64
	16	49° 50′	7° 15′	0·56
	17	49° 50′	6° 00′	0·54
21. iii. 55	18	49° 53′	5° 12′	0·58

of this phosphate-rich water, Dr L. H. N. Cooper in R.V. *Sarsia* made a survey of the area at the mouth of the English Channel, working twenty stations as shown in Fig. 4 between 14 and 21 March 1955. Table 3 gives the positions of the stations and phosphate concentrations found at 10 m, and areas characterized by differing phosphate concentrations are indicated by shading in the figure.

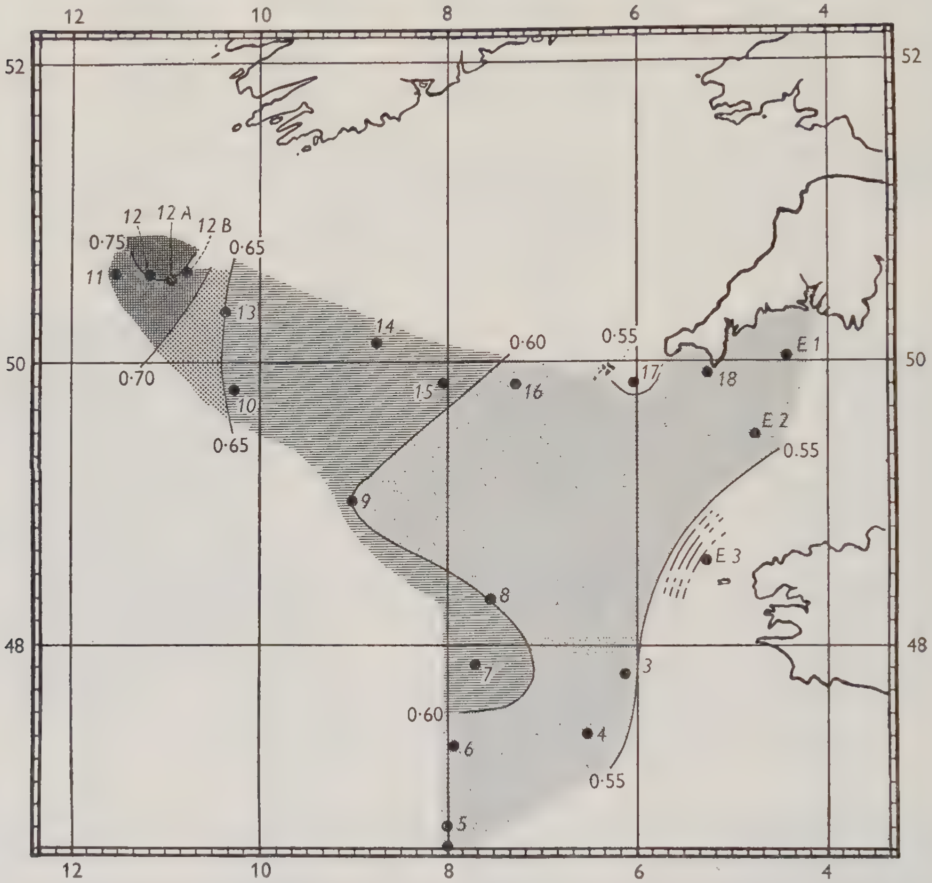

Fig. 4. Distribution of phosphate at 10 m depth in part of Western Approaches, 14–21 March 1955. Contour lines at 0·05 μg atom P/l. intervals.

Although notably high phosphate concentrations were found in surface waters over the continental slope southwest of Ireland there is no evidence that water from there has ever entered the English Channel, and no satisfactory source for the high phosphate water was found within the area surveyed. Low values of around 0·05 μg atom P/l. were found in the upper 10 m layer in July and August.

The vertical distribution of phosphate is shown in Fig. 2, and it will be seen that the water column was almost homogeneous until 9 May, by which date plant growth had removed a considerable amount of phosphate from the upper 25 m. Low concentrations of phosphate in the upper layers persisted until the breakdown of the thermocline.

Silicate

The vertical distribution of silicate is shown in Fig. 3 It is seen that the water column was homogeneous up to and including 9 May. On that date there was little variation in silicate concentration with depth, in contrast to the marked layering of phosphate. Silicate was low throughout the water column. During the summer, silicate was less in the layer above the thermocline than in the deeper water, but no very low concentrations were recorded, the smallest being around 0·2 μg atom Si/l. in the upper 20 m on 13 July.

Silicate at these depths had increased to about 1·5 μg atom Si/l. on 11 August, although the intervening period had been notable for bright warm weather (as recorded above) such as would be expected to favour growth of diatoms and consumption of silicate. It has already been observed that in the deeper water at least, a significant change in salinity occurred between these dates.

By 18 October silicate concentration was uniform throughout the water column, and was higher than at the beginning of the year. Mean values for December are lower than for November, which is unexpected.

Integral mean concentrations

The computed figures are given in Table 2. The decreases in the spring representing consumption of nutrients by the phytoplankton were: phosphate 0·42 μg atom P/l., 'total phosphorus' 0·40 μg atom P/l., silicate 2·43 μg atom Si/l.

SUMMARY

Temperatures and salinity, phosphate, total phosphorus and silicate analyses of water from the International Hydrographic Station E1 during 1955 are discussed. The seasonal variation is shown, and it appears that consumption of nutrients by plants in the spring was: phosphate 0·42 μg atom P/l., 'total phosphorus' 0·40 μg atom P/l., silicate 2·43 μg atom Si/l., these being means for the whole water column. Some irregularities are pointed out; they are probably attributable to changes in the water mass.

REFERENCES

Armstrong, F. A. J. 1954. Phosphorus and silicon in sea water off Plymouth during the years 1950 to 1953. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 381–92.

—— 1955. Phosphorus and silicon in sea water off Plymouth during 1954. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 223–8.

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ON THE BEHAVIOUR OF BARNACLES

III. FURTHER OBSERVATIONS ON THE INFLUENCE OF TEMPERATURE AND AGE ON CIRRAL ACTIVITY

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(Text-figs. 1–3)

The results of investigations into the relation between temperature and cirral activity in certain barnacles were described in Part I of this series (Southward, 1955*a*). It was shown that in each of five species commonly found on the shore in Britain, the range of temperature over which the cirri were active and the temperature at which this activity was greatest could be related to the geographical distribution of the species and to the temperatures experienced in the normal habitat. In a further paper it was shown that in one of these species (*Chthamalus stellatus*) the frequency of beating was apparently lower in older individuals (Southward, 1955*b*). These results have been confirmed and extended by experiments made on a further five species of barnacles from a wider range of habitats (Table 1).

TABLE 1. SPECIES OF BARNACLES INVESTIGATED

Species	Where collected	Tide-level or depth	Speed of water current in apparatus
Lepas anatifera L.	Growing on cork lifebelt, found off Plymouth	—	Nil
Balanus improvisus Darwin	Upper reaches of Tamar estuary, near Weir Quay	L.W.N.	0·5 cm/sec, with occasional bursts of 5–10 cm/sec
B. amphitrite Darwin var. *denticulata* Broch	On piles near outflow of warm water from power station, Plym estuary	L.W.N.	As *B. improvisus*
B. balanus L.	On shells of *Modiolus* from Anglesey	*ca.* 12 fathoms	As *B. improvisus*
Hexelasma hirsutum Hoek	Continental slope in vicinity of 48° 33′ N., 10° 4′ W.	570–770 fathoms	4–16 cm/sec

All temperatures are quoted in degrees Centigrade. Climatic details have been taken from the following references: Admiralty, 1946; Air Ministry, 1949; International Council, 1933.

I am indebted to Dr D. J. Crisp, who supplied living *Balanus balanus*, for advice in this work and to Mr F. G. C. Ryder for construction of apparatus. The experiments were carried out during the tenure of a D.S.I.R. Senior Research Award.

EXPERIMENTAL CONDITIONS

The experimental conditions differed slightly from those of the earlier work. The animals were observed in a long trough of Perspex, divided by a central partition. The trough was filled with filtered offshore water, which was replenished from time to time. All water movement was set up by means of an enclosed paddle wheel, also of Perspex, placed at one end of the trough and driven by belt and pulleys from an electric motor.

The temperature of the water in the trough was raised or lowered by immersing in it small vessels containing hot water or ice. During the duration of each experiment the temperature was easily controlled to within 0·5°, the temperature limits adopted in the previous work. However, for temperatures below 4° some difficulty was experienced because of warming by the air during the long period of observation necessitated by the slow frequency of beating at low temperatures, and a smaller number of barnacles was placed in a finger bowl standing in ice water or freezing mixture. Under these circumstances water movement was set up by a jet of compressed air.

In the trough, the water currents could be controlled by means of a resistance in series with the electric motor. The current speed was measured approximately by timing the movement of small particles in the water. As previously, the frequency of beating was assessed by noting the time taken for ten complete openings and closings of the valves accompanied by partial or complete protrusion of the cirri. The values so obtained were converted to the number of beats per 10 sec. Approximately ten specimens were examined at each temperature. The temperature intervals were about 4° or 5° and the rate of heating or cooling was adjusted to 4°/h.

Except for *Balanus amphitrite* (see below) all specimens were observed while still attached to small pieces of the substratum.

Only *B. improvisus* and *B. amphitrite* were examined within 24 h of collection; *B. balanus* was sent by post and nearly 7 days elapsed between collection and examination; *Lepas* was examined after an unknown period out of water followed by nearly 24 h in the aquarium water at Plymouth; *Hexelasma* was brought back on R.V. *Sarsia* under running sea water and examined within 7 days of being dredged up. Chances of acclimatization to temperatures other than those normally experienced must therefore be allowed for in interpreting the results.

RESULTS

Lepas anatifera (Fig. 1; Table 3)

All specimens of *Lepas* at times showed a tendency to hold the cirri extended (extension response—see Part I), even in still water. The rate of water movement had no observable effect on the proportion of individuals beating, and the actual measurements were made without a current. It was necessary to observe a large number of specimens and choose those that were showing rhythmic beating.

Some beating was noted below 1·5°, although there was almost instantaneous and complete chill coma on lowering the temperature to 0·5°. The mean frequency of beating increased almost linearly between 3·8° and 19·8°, from 0·55 to 2·85 beats per 10 sec. Above 20° the frequency slowed down, and all beating ceased at 33°. More than half the specimens succumbed to heat coma at 33·5°.

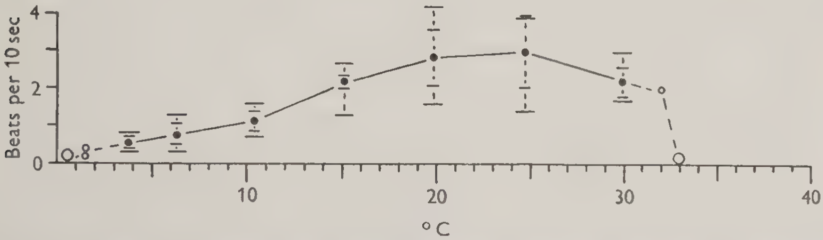

Fig. 1. *Lepas anatifera*: frequency and range of beating of the cirri. The larger circles denote absence of beating, and the smaller circles single observations; the dots indicate the mean frequency at each temperature, while the large and small cross-lines mark, respectively, the range and standard deviation of the samples on either side of the mean.

Both the optimum cirral activity and the position of maximum cirral frequency shown by these specimens occur at relatively low temperatures for a species which has been recorded from all the oceans (see Darwin, 1854). There is, therefore, a strong possibility that the species contains physiological varieties adapted to different temperature regimes. Certainly, these specimens would be ill-suited to temperature conditions prevailing in the Atlantic north of Nova Scotia and North Norway, or south of Cape Cod and Gibraltar.

Balanus improvisus (Fig. 2A; Table 4)

Specimens of *B. improvisus* showed cirral activity over a wide range of temperatures. Between 8·8° and 20° the mean frequency increased uniformly from 2 to over 8 beats/10 sec, and a maximum was reached at 30°. Above 30° the frequency of beating declined sharply, and beating was not detected above 35·5°.

The behaviour at low temperatures was most interesting. There was a change in the temperature coefficient at about 8–9°, and the mean frequency of beating declined only slowly as the temperature was lowered. Most specimens continued to beat rhythmically down to 4°, and one or two continued to do so down to –2°, the lowest temperature tested. Even after remaining at this low temperature for 10 min, less than half the specimens (4 out of 10) showed chill coma.

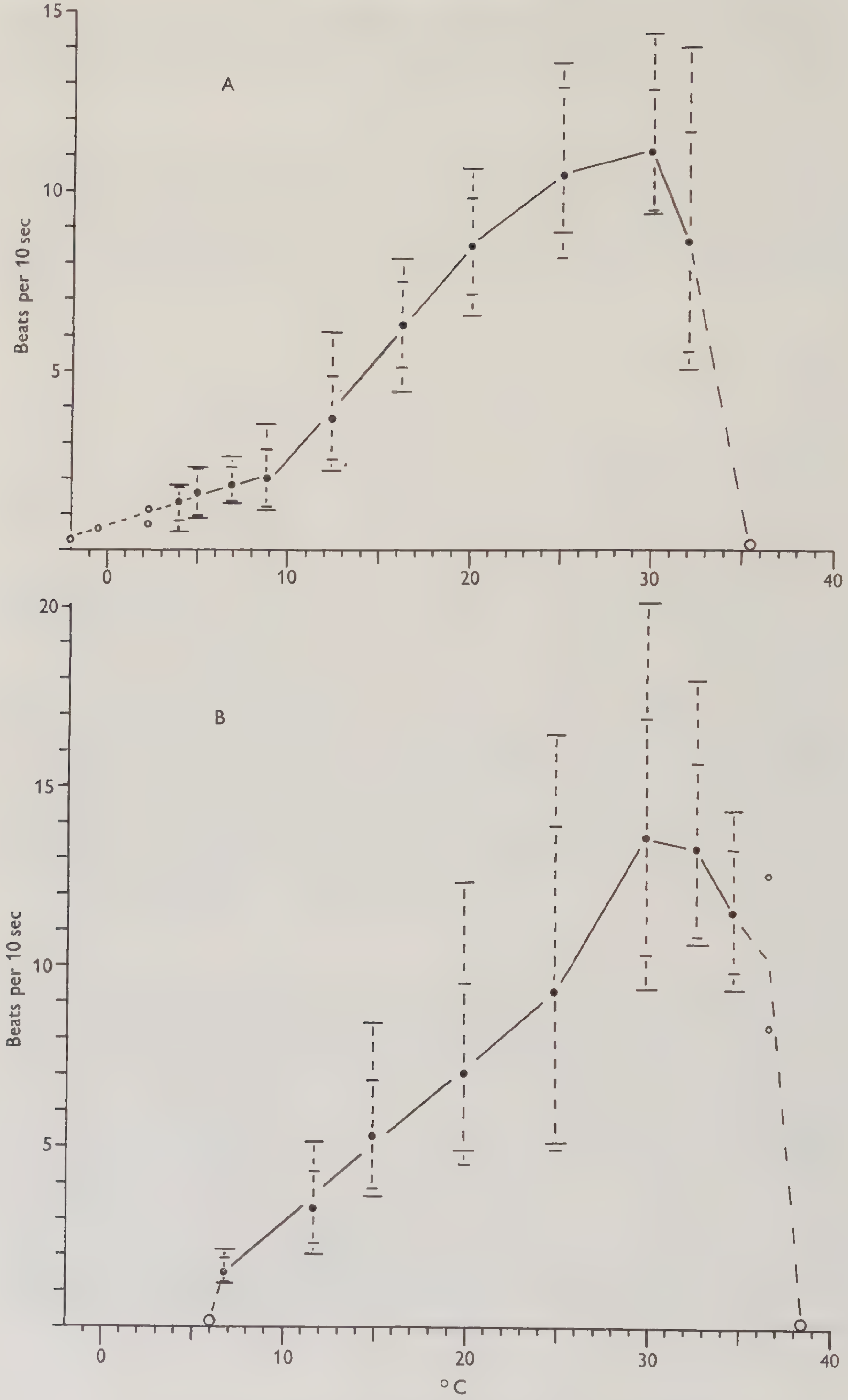

Fig. 2. The frequency and range of beating of the cirri of (A) *Balanus improvisus*, (B) *B. amphitrite*. For explanation of symbols refer to Fig. 1.

The wide range of optimum temperature in this species is in accordance with its wide geographical range; it has been recorded from Nova Scotia and the Baltic, and extends through the tropics to southern South America (Darwin, 1854; Pilsbry, 1916; Segerstråle, 1953). Nevertheless, the position of the maximum cirral frequency is more characteristic of a tropical species.

Balanus amphitrite (Fig. 2B; Table 5)

Only twelve specimens of *B. amphitrite* were collected with their calcareous bases intact; they were mounted on glass slides with SIRA wax before being placed in the trough. A few specimens showed the fast type of beat at some temperatures; they have not been included in the results. At most temperatures it was possible to measure the cirral frequency of nine specimens. Below 10° only five individuals were active, and beating was not observed below 6°. Between 6·8 and 24·9° the mean frequency of beating increased linearly from 1·5 to 9·3 beats/10 sec. The maximum frequency was reached at 29·9°, with a mean of 13·5 beats/10 sec. Beating slowed down slightly between 30° and 36°, and ceased completely at 38·4°.

The high upper limit to the temperature range, and the relative ease with which beating was carried on above 30°, agree well with the predominantly tropical and warm temperate distribution of the species. Its normal northern limit appears to be northern Spain (Fischer-Piette, 1955), and beyond this it is found only in docks, harbours or estuaries that are artificially warmed (see Bishop, 1950; Crisp & Molesworth, 1951). At Plymouth after a warm summer I have found young specimens on the shore of the Sound, as well as in the Plym estuary, but the adults survive only in the vicinity of the warm water outlet from the power station. The absence of beating below 6°, and the difficulty of obtaining beating between this temperature and 10° suggest that little, if any, acclimatization has taken place in the local population.

Balanus balanus (Fig. 3; Table 6)

The specimens of *B. balanus* sent by Dr Crisp consisted of four groups. The first three groups consisted of freshly collected specimens showing respectively one, two and three growth rings; the fourth group of specimens with two or three growth rings had been kept in the laboratory at Menai Bridge for 4 months without food. *B. balanus* is the only British barnacle that has been found to show clear growth marks, and the groups with one, two and three rings correspond to individuals in their second, third and fourth years since settlement (Crisp, 1954).

The behaviour of each group was noted separately, but as each group contained less than ten specimens and only the two- and three-ring groups were active at all temperatures, the results from these groups have been combined to show the effect of temperature on cirral activity. For this purpose specimens showing the fast type of beat have been ignored. One specimen showed signs of beating at −2°, but none was active below this temperature. Above zero the frequency of beating increased fairly linearly, from between 1·1 and 1·3 beats/10 sec at 1·9° to a maximum of 4·8 beats at 20·2°. Above 20° the frequency of beating declined, at first slowly up to 26°, then more rapidly; beating ceased at 30°.

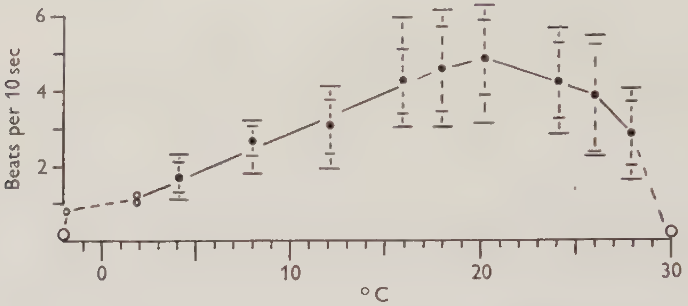

Fig. 3. *Balanus balanus*: frequency and range of beating of the cirri. For explanation of symbols refer to Fig. 1.

This species is predominantly of Arctic and Boreal distribution (Darwin, 1854; Weltner, 1900), and it seems doubtful whether it ever occurs south of the Bristol Channel and North Sea (Crisp & Southward, 1957). Thus in its normal habitat it is hardly likely to be subjected to sea temperatures above 17°; even when it occurs on the shore it is found in places well protected from the sun and the air, such as beneath *Ascophyllum* (Crisp, 1954) or in crevices and under stones (personal records). The species, therefore, is not operating at the extreme upper limit of its optimum range, unlike the other northern form *B. balanoides* (Southward, 1955*a*).

The effect of age on cirral activity in this species is shown in Table 2. The range and the mean of the cirral frequency is given for each group at four temperatures; at other temperatures one or more of the groups failed to beat the cirri. It can be seen that the younger barnacles had the highest frequency of cirral beat, and that the cirri beat progressively more slowly in the older groups. The lowest frequency of all was found in the starved specimens; except at 4° the differences between these specimens and all the others were greater than between any two age-groups of the fresh specimens.

The differences between the means are not very great for such small samples and statistically they are hardly significant. For example, in tests by the t distribution that the one-ring and three-ring groups belonged to the same statistical population, the value of P varied from 0·05 to 0·1.

However, the evidence tends to support the earlier observation that ageing in barnacles is accompanied by a slowing down of the cirral activity. It seems that starvation has a similar effect. Probably there is a depression of the metabolic rate in both old and in starved individuals.

These differences relate to the ordinary rhythmic beat. A fast type of beat was shown by a few individuals at some temperatures, but only among the youngest specimens and in the starved group. These are the groups in which the fast type of beat would be expected if it was largely a feeding reaction (cf. Southward, 1955*b*).

Further differences between the four groups of *B. balanus* were noticed at the upper limit of temperature for cirral activity. All starved specimens ceased beating at 27·9°, all three-ring and two-ring specimens at 29° and 30° respectively, while more than half the youngest group continued active to 30·5°. These differences are of the same order as those of cirral frequency.

TABLE 2. CIRRAL BEHAVIOUR IN THE DIFFERENT AGE-GROUPS OF *BALANUS BALANUS*

Temperature (° C)	Age-group	No. tested	No. showing ordinary beat	Frequency of ordinary beat			No. showing fast beat	No. not beating
				Mean	Range	S.D.		
4·0	1 ring	7	3	2·333	2·0–2·8	±0·417	0	4
	2 ring	7	4	1·950	1·6–2·3	±0·310	0	3
	3 ring	5	3	1·366	1·1–1·7	±0·307	0	2
	Starved	5	2	0·950	0·7–1·2	±0·353	1	2
8·0	1 ring	7	6	2·883	2·4–3·3	±0·338	0	1
	2 ring	7	6	2·816	2·5–3·2	±0·260	0	1
	3 ring	5	4	2·40	1·8–2·8	±0·454	0	1
	Starved	5	5	1·140	0·9–1·5	±0·251	0	0
15·9	1 ring	7	3	4·833	4·1–5·2	±0·637	0	4
	2 ring	7	6	4·583	3·4–5·9	±0·855	0	1
	3 ring	5	5	3·760	3·0–4·8	±0·695	0	0
	Starved	5	4	2·775	1·6–3·4	±0·801	1	0
20·2	1 ring	7	3	5·433	4·8–6·2	±0·711	3	1
	2 ring	7	5	4·920	3·9–5·9	±0·837	0	2
	3 ring	5	5	4·70	3·1–6·2	±1·332	0	0
	Starved	5	4	3·075	2·3–3·7	±0·684	1	0

Hexelasma hirsutum

So far, only a preliminary investigation has been made into the behaviour of *Hexelasma*. Of twenty-four specimens tested in the trough none could be induced to show rhythmic beating of the cirri under any variation of temperature, water movement or illumination. With a water current from 4 to 16 cm/sec an extension response was given by up to eight specimens simultaneously at temperatures between 2·7° and 8°. Below 2·7° and down to –2° one or two specimens showed very slight movements of the valves and reacted to touch by closing. Above 8° a few specimens showed what has been called the pumping type of beat (Southward & Crisp, 1957). The cirri were not protruded, but minor movements of the prosoma and valves caused a current of water to enter the mantle cavity near the mouth; this water passed through the mantle cavity and was ejected in puffs behind the prosoma (see Crisp & Southward, 1956). The frequency of these exhalant puffs increased from 0·05 and 0·06/10 sec at 8·5° to 0·10 and 0·18/10 sec at 19°. The temperature was not taken higher than 19° to avoid risk of damage to the specimens, but even at this temperature there were signs of jerky movements similar to those that precede heat coma in other barnacles.

Hexelasma hirsutum has been recorded elsewhere from the Faroe-Shetland Ridge (59° 40′ N., 7° 21′ W.) at 516 fathoms (Hoek, 1883) and from the Azores (38° 31′ N., 26° 49′ W.) at 465 fathoms (Gruvel, 1920). The bottom-water temperatures at all localities at which the species has been found, or at nearby stations of similar depth, vary from 7° to 9° (Hoek, 1883; Rouch, 1948; Cooper, 1952) and the annual variation is probably less than 1° (Dr L. H. N. Cooper, personal communications). As far as cirral extension is concerned, the specimens of *Hexelasma* examined were thus living very close to the upper limit of their optimum range. Probably the species can live in colder and deeper water when other conditions allow.

The absence of rhythmic beating within the optimum range suggests that respiratory needs are satisfied by the passage of water over the branchiae caused by the twisting movements of the extended cirri, by the occasional withdrawing movements of the cirri, or by external water currents. The pumping beat at higher temperatures suggests the onset of respiratory difficulties; the low frequency of the pumping movements may indicate lack of co-ordination for rhythmic activity.

DISCUSSION

In three of the five species dealt with in this Part, namely *Balanus improvisus*, *B. amphitrite* and *B. balanus* the range of cirral activity is closely related to the geographical distribution.

The behaviour of *B. amphitrite*, and, in the upper part of its range, *B. improvisus* is similar to that of one of the two southern species discussed in Part I, *B. perforatus*. These three species belong to the same subgroup, or closely related subgroups, of the genus *Balanus* (Darwin, 1854; Pilsbry, 1916); the resemblance in behaviour is not therefore unexpected. *B. improvisus* is the only species of the group to occur in genuinely cold climates, and the form of its behaviour suggests that it is a tropical species that has been able to extend the lower end of its range of optimum temperature. This adaptation may be connected with the remarkable euryhalinity shown by the species (Darwin, 1854; Pilsbry, 1916). It seems probable that a more flexible metabolism is needed to cope with the osmoregulatory requirements of a brackish water habitat, and this may have facilitated tolerance of a wider range of temperatures.

The range of optimum temperature in *B. balanus*, and the temperature at which maximum cirral frequency was shown, differ considerably from those of the related species, *B. crenatus*, investigated previously (Part I). There is, in fact, more resemblance between *B. balanus* and *B. balanoides*. All three species are of generally northern distribution, but their exact southern limits vary. As stated, *B. balanus* does not apparently occur as far south as the English Channel, where *B. crenatus* has its southern limit; *B. balanoides* is present in the Bay of Biscay (Southward & Crisp, 1956) and in a limited area of north-west Spain (Fischer–Piette & Prenant 1956). The order of increasing tolerance of high temperatures is *B. crenatus–B. balanus–B. balanoides*, while the position of maximum cirral frequency increases *B. balanoides–B. balanus–B. crenatus*. There is thus no exact relation between the extreme southern limits of the species and their temperature tolerances for cirral activity. No doubt other environmental factors besides temperature affect the limits of the species.

The temperature relations of *Lepas* and *Hexelasma* have already been discussed (pp. 325 and 330). The behaviour of the latter species reflects the relatively uniform temperatures experienced in deep water compared with the intertidal or shallow water habitats favoured by the species of *Balanus*.

SUMMARY

The range of temperature over which the cirri were active, and the frequency of beating of the cirri at different temperatures were measured in a further five species of barnacles from a variety of habitats. In three of the species the temperature range and frequency of cirral beat were related to the geographical distribution of the species. The tropical and warm temperate species *Balanus amphitrite* was active at higher temperatures, and showed a greater frequency of beating than the northern species *B. balanus*; conversely, the latter was active to much lower temperatures than *B. amphitrite*. The species with the widest geographical range, *B. improvisus*, showed cirral activity over the widest range of temperatures, although its behaviour at high temperatures was similar to that of the related species *B. amphitrite*. It is suggested that *B. improvisus* is a tropical species that has adapted itself to colder climates; its tolerance of a wide range of temperatures may be associated with its tolerance of low salinities.

The stalked barnacle *Lepas anatifera* showed too restricted a temperature range for its supposed world-wide distribution, and it is suggested that the species may contain physiological races adapted to different climates. The extremely restricted range of temperatures over which the cirri of the deep-sea barnacle *Hexelasma hirsutum* were active can be correlated with the almost uniform temperature conditions at great depths.

In *B. balanus* age-groups can be clearly distinguished by growth rings on the shell, and the cirral frequency was found to be slower in the older specimens. Even slower cirral beating was found in some starved specimens.

REFERENCES

Admiralty, London, 1946. *World Climatic Charts*, Sheets I and II.

Air Ministry, London, 1949. *Monthly Sea Surface Temperatures of North Atlantic Ocean.*

Bishop, M. W. H., 1950. Distribution of *Balanus amphitrite* Darwin var. *denticulata* Broch. *Nature, Lond.*, Vol. 165, p. 409.

Cooper, L. H. N., 1952. The physical and chemical oceanography of the waters bathing the continental slope of the Celtic Sea. *J. mar. biol. Ass. U.K.*, Vol. 30, pp. 465–510.

Crisp, D. J., 1954. The breeding of *Balanus porcatus* (Da Costa) in the Irish Sea. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 473–96.

Crisp, D. J. & Molesworth, A. H. N., 1951. Habitat of *Balanus amphitrite* var. *denticulata* in Britain. *Nature, Lond.*, Vol. 167, p. 489.

Crisp, D. J. & Southward, A. J., 1956. Demonstration of small scale water currents by means of milk. *Nature, Lond.*, Vol. 178, p. 1076.

—— —— 1957. The distribution of some common intertidal organisms along the coasts of the English Channel. (In preparation.)

Darwin, C., 1854. *A Monograph on the Sub-Class Cirripedia: Balanidae, Verrucidae, etc.* 684 pp. London: Ray Soc.

Fischer-Piette, E., 1955. Répartition, le long des côtes septentrionales de l'Espagne, des principales espèces peuplant les rochers intercotideaux. *Ann. Inst. océanogr. Monaco*, T. 31, pp. 37–124.

Fischer-Piette, E. & Prenant, M., 1956. Distribution des cirripèdes intercotideaux d'Espagne septentrionale. *Bull. Centre Études et Recherches scientifiques Biarritz.* T. 1, pp. 7–19.

Gruvel, A., 1920. Cirrhipèdes. *Result. Camp. sci. Monaco*, Fasc. 53, 88 pp.

Hoek, P. P. C., 1883. Cirripedia. *Challenger Reports, zool.*, Vol. 8, Pt. 25, 169 pp.

International Council, 1933. *Atlas de température et salinité de l'eau de surface de la Mer du Nord et de la Manche.* Copenhagen.

Pilsbry, H. A., 1916. The sessile barnacles (Cirripedia) contained in the collections of the U.S. National Museum. *Bull. U.S. nat. Mus.*, Vol. 93, pp. 1–366.

Rouch, J., 1948. Stations hydrologiques. *Result. Camp. sci. Monaco*, Fasc. 108, 26 pp.

Segerstråle, S., 1953. Further notes on the increase in salinity of the inner Baltic and its influence on the fauna. *Comment. biol., Helsingf.*, Vol. 13, No. 15, pp. 1–7.

Southward, A. J., 1955*a*. On the behaviour of barnacles. I. The relation of cirral and other activities to temperature. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 403–22.

—— 1955*b*. On the behaviour of barnacles. II. The influence of tide-level and habitat on cirral activity. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 423–33.

Southward, A. J. & Crisp, D. J., 1956. Fluctuations in the distribution and abundance of intertidal barnacles. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 211–29.

—— —— 1957. On the behaviour of barnacles. IV. Types of cirral activity and their relation to feeding and respiration. (In preparation.)

Weltner, W., 1900. Die Cirripedien der Arktis. *Fauna Arctica*, Bd. I, pp. 287–312.

APPENDIX

TABLE 3. FREQUENCY OF BEATING OF THE CIRRI OF *LEPAS ANATIFERA*

From off Plymouth, 1. x. 56; examined 2. x. 56

Temperature (° C)	Frequency of beating, as beats per 10 sec		
	Mean	Range	S.D.
0·5	—	Nil	—
1·5	2 specimens only	0·2, 0·4	—
3·8	0·557	0·3–0·8	±0·161
6·3	0·770	0·3–1·3	±0·292
10·4	1·130	0·7–1·6	±0·283
15·1	2·20	1·3–2·7	±0·125
19·8	2·850	1·6–4·2	±0·760
24·7	2·50	1·4–2·9	±0·454
29·7	2·185	1·7–3·0	±0·422
32·0	1 only	2·0	—
33·0	—	Nil	—

TABLE 4. FREQUENCY OF BEATING OF THE CIRRI OF *BALANUS IMPROVISUS*

From the Tamar estuary, 13. ii. 56 and 15. ii. 56

Temperature (° C)	Frequency of beating, as beats per 10 sec		
	Mean	Range	S.D.
−2·0	1 only	0·3	—
−0·5	1 only	0·6	—
2·3	2 only	0·7, 1·1	—
4·0	1·344	0·5–1·8	±0·470
5·0	1·595	0·9–2·3	±0·703
6·9	1·833	1·3–2·6	±0·477
8·8	2·018	1·1–3·5	±0·778
12·4	3·677	2·2–6·1	±1·171
16·2	6·266	4·4–8·1	±1·180
20·0	8·440	6·5–10·6	±1·318
25·0	10·420	8·8–13·5	±2·360
30·0	11·057	9·3–14·3	±1·674
32·0	8·550	5·0–13·9	±3·078
35·5	—	Nil	—

TABLE 5. FREQUENCY OF BEATING OF THE CIRRI OF *BALANUS AMPHITRITE*

From the Plym estuary, 9–10. ii. 56

Temperature (° C)	Frequency of beating, as beats per 10 sec		
	Mean	Range	S.D.
6·0	—	Nil	—
6·8	1·520	1·2–2·1	±0·311
11·7	3·322	2·0–5·1	±0·983
14·9	5·30	3·6–8·4	±1·495
19·9	6·988	4·9–12·3	±2·467
24·9	9·337	5·1–16·4	±4·547
29·9	13·562	9·3–20·0	±3·271
32·7	13·20	10·6–17·9	±2·371
34·7	11·487	9·3–14·3	±1·739
36·7	2 only	8·3, 12·5	—
38·4	—	Nil	—

TABLE 6. FREQUENCY OF BEATING OF THE CIRRI OF *BALANUS BALANUS*

From Anglesey, examined 17–18. iii. 56: 2-ring and 3-ring

Temperature (° C)	Frequency of beating, as beats per 10 sec		
	Mean	Range	S.D.
-2·0	1 only	0·8	—
1·9	2 only	1·1, 1·3	—
4·0	1·70	1·1–2·3	±0·420
8·0	2·650	1·8–3·2	±0·389
12·1	3·044	1·9–4·1	±0·731
15·9	4·209	3·0–5·9	±0·862
18·0	4·544	3·0–6·1	±1·115
20·2	4·810	3·1–6·2	±1·009
24·1	4·211	2·8–5·6	±1·003
26·0	3·775	2·3–5·4	±1·563
27·9	2·80	1·6–4·0	±0·868
30·0	—	Nil	—

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VIABILITY AND GLYCOGEN RESERVES IN THE NEWLY LIBERATED LARVAE OF *OSTREA EDULIS* L.

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It has been suspected that broods of larvae of the European flat oyster, *Ostrea edulis* L., differ considerably in their vigour and in the ease with which they can be reared in the laboratory (Cole, 1939; Bruce, Knight & Parke, 1940; Walne, 1956). The discovery of the cause of this phenomenon would be a step forward in breeding experiments, and might possibly indicate a way in which the vigour of larvae could be improved. It was thought that a possible explanation might be differences in the glycogen reserves in the larvae at the time of liberation, and for this reason the series of analyses reported in this paper were made.

Method

Twelve broods of larvae were obtained from oysters kept in running sea water at the Conway laboratory. A sample of each brood, in most cases between 100,000 and 200,000 larvae, was washed with distilled water, dried and preserved for glycogen assay. The remainder of the brood was reared by Mr P. R. Walne at Conway by his standard technique (Walne, 1956). Briefly, this method involves placing about 1000 larvae in 1 l. of filtered sea water in a glass vessel, which is stood in a water bath at 20-22° C. The larvae are kept stirred by gentle aeration, and in these experiments were fed with cultures of the flagellate *Isochrysis galbana*, the density of which was kept at about 50 cells per mm^3. When eyed larvae were first observed collectors in the form of mussel shells or broken unglazed saucers were added. When all the larvae had either metamorphosed on collectors or were clearly moribund, the experiment was concluded and the number of metamorphosed larvae counted. The twelve broods which were tested gave a considerable range of results, the percentage of larvae metamorphosing ranging from 0 to 25.

It was desirable to be able to express the glycogen content as a percentage of organic matter, so as to eliminate differences due to the relative proportion of weight represented by the physiologically inert calcareous shell. The following procedure fulfilled this requirement, and at the same time eliminated errors due to sea-salt contamination. To each sample of larvae 1·2 N hydrochloric acid was added drop by drop until no more effervescence occurred and a slight excess of acid was present. The acid suspension was transferred to the central compartment of a Perspex electrodialyser, the basis of which were Permutit ion-exchange membranes 'Permaplex' A-10 and C-10. This apparatus was similar to that described by Blainey & Yardley (1956), differing in that an iron cathode and a carbon anode were used; and instead of acid and alkali respectively in the electrode compartments, a continuous flow of tap water removed the products of electrolysis. The dialyser was connected to a 12 V battery overnight. Preliminary tests showed this to demineralize such a solution beyond the limits of weighing, but an 8 h period was insufficient. Leakage of solution from the compartment could be prevented by the careful use of vaseline on the Perspex surfaces gripping the membranes.

The suspension was transferred to a tared tube and dried at 105° C to constant weight, which ranged in the samples from 25 to 90 mg. Hydrolysis of glycogen was carried out in the same tube, 10 ml. of 3% (v/v) sulphuric acid being added. The tubes were immersed in a boiling-water bath for 3 h, and early in this operation the dried material was shaken into a good suspension in the acid. The samples were neutralized, filtered, and, with rinsings, made up to either 100 ml. or 25 ml. according to the weight available. Duplicate 10 ml. aliquots were taken, and reducing sugars determined according to the Hagedorn-Jensen method as modified by Hulme & Narain (1931).

TABLE 1. PARTICULARS OF OYSTER LARVAE

The date of liberation, parentage, size and glycogen content of twelve broods of oyster larvae collected at Conway in 1955. The parent stock were all brought in from the oysterage on 9 July. The 'Conway' oysters were 3-year-old oysters bred in the Conway tanks. The 'Brittany' oysters were imported from France in 1954 and relaid in the Tal-y-foel oysterage. The larval size is expressed as the percentage in each size category, the length of the shell being the dimension measured. The actual number of spat obtained in each experiment is shown and this number is also expressed as a percentage of the initial number of larvae in the experiment. The final column gives the glucose found expressed as a percentage of the dry organic matter (to convert to glycogen multiply by 0·927).

Date of liberation	Oyster	Percentage size distribution of larvae at liberation (μ)						Number of spat	Glucose (%)
		150–159	160–169	170–179	180–189	190–199	200–209		
16 July	Conway	—	—	4	52	28	16	0	15·7
20	Conway	8	28	40	24	—	—	0	15·0
25	Conway	—	8	52	40	—	—	0	16·6
26	Brittany	—	32	40	20	8	—	0	15·8
27	Brittany	—	—	28	72	—	—	134 (25%)	15·8
29	Brittany	—	32	56	12	—	—	0	16·6
30	Brittany	—	4	44	48	4	—	42 (7%)	14·9
22 Aug.	Brittany	4	4	40	52	—	—	0	14·5
23	Conway	4	72	20	4	—	—	139 (17%)	15·3
23	Conway	56	44	—	—	—	—	33 (5%)	16·0
24	Brittany	4	36	52	8	—	—	0	15·7
25	Brittany	4	44	48	4	—	—	0	15·1

Conclusion

The amounts of glucose found, expressed as a percentage of organic matter, lay in all twelve cases between 14·5 and 16·6 (equivalent to 13·4 and 15·4% glycogen). The details are shown in Table 1. In view of such very small differences in glycogen reserves, it was concluded that some other factor must be responsible for the large viability differences observed. Certainly no correlation can be observed between the glycogen reserves and either the size of the larvae at liberation or the yield of spat.

SUMMARY

Samples of twelve broods of oyster larvae were subjected to a standard laboratory rearing technique while other samples were assayed for glycogen. The glycogen reserves of the larvae at liberation lay between 13·4 and 15·4% of the dry organic matter. No correlation was observed between the glycogen reserve and either the size of the larvae at liberation or the yield of spat in the rearing experiments.

REFERENCES

Blainey, J. D. & Yardley, H. J., 1956. Electrolytic desalting with ion-exchange membranes. *Nature, Lond.*, Vol. 177, p. 83.

Bruce, J. R., Knight, M. & Parke, M. W., 1940. The rearing of oyster larvae on an algal diet. *J. mar. biol. Ass. U.K.*, Vol. 24, pp. 337–74.

Cole, H. A., 1939. Further experiments in the breeding of oysters (*Ostrea edulis*) in tanks. *Fish. Invest., Lond.*, Ser. 2, Vol. 16, No. 4.

Hulme, A. C. & Narain, R., 1931. The ferricyanide method for the determination of reducing sugars. A modification of the Hagedorn-Jensen-Hanes technique. *Biochem. J.*, Vol. 25, pp. 1051–61.

Walne, P. R., 1956. Experiments on rearing the larvae of *Ostrea edulis* L. in the laboratory. *Fish. Invest., Lond.*, Ser. 2, Vol. 20, No. 9.

J. mar. biol. Ass. U.K. (1957) 36, 339–375
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THE BREEDING AND FECUNDITY OF THE LONG ROUGH DAB *HIPPOGLOSSOIDES PLATESSOIDES* (FABR.) AND THE ASSOCIATED CYCLE IN CONDITION

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(Text-figs. 1–11)

Although the Long Rough Dab, *Hippoglossoides platessoides* (Fabr.), is represented by two subspecies, *H. p. limandoides* (Bloch) round the northern European coasts and *H. p. platessoides* on the North American Atlantic seaboard (Norman, 1934) and is often very abundant, little is known about its breeding biology.

The American race has been discussed by Huntsman (1918), and Bigelow & Schroeder (1953), but they do not consider the breeding biology in any detail. For the European race Saemundsson (1908, 1925, 1927) for Icelandic, Krüger (1942) for Baltic, Essipow & Slastnikow (1932) and Milinsky (1944) for Barents Sea fish, refer briefly to the breeding habits. Notes on the occurrence of Long Rough Dab eggs are scattered through the literature (Bal, 1943; Williamson, 1899; Günther, 1888; Rass, 1936; Otterstrøm, 1906; Pertseva, 1939 and others). As to fecundity, Fulton (1891) and Mitchell (1913) have given egg counts for five and eleven fish respectively from British coasts, and Milinsky (1944) for three fish from the Barents Sea.

In this paper the breeding biology of the Clyde population of Long Rough Dabs will be described. Since many aspects of breeding phenomena are closely associated with the condition of the fish, this is also considered (see also Fulton, 1904). Lastly, the fecundity of the species will be considered in relation to the age, length and weight of the fish. Some of these subjects are treated statistically, and details of the analyses, together with discussions of their validity, are given separately in Appendices 1–4. The biological significance of the analyses are given along with the other results of the investigation in the first part of the paper. The growth rate of some of the fish has been considered in previous papers (T. B. Bagenal, 1955*a*, *b*).

My special thanks are due to Mrs Jean Morrison who counted the eggs for the fecundity estimates, Miss Sheila Morris, who did most of the calculations, my wife for advice on statistical treatments, and the officers and crews of the Marine Station's research vessels for the collection of the material.

METHODS AND MATERIALS

The material on which this paper is based was caught in approximately monthly samples taken from October 1953 to May 1955. The dates of capture are given in Table 1. The fish ranged from 5 to 33 cm in length and 0 to 7+ years of age. The samples were obtained with a small-mesh cotton V.D. trawl of the following dimensions: headline, 49 ft.; footrope, 78 ft.; bridles, 15 fathoms; lower wings and belly mesh size, $2\frac{1}{2}$ in. bar, all other meshes, 1 in. bar. It is thought that this trawl took an adequate sample of the population, though the mesh-size selection gave noticeably biased samples of the younger age-groups (see T. B. Bagenal, 1955*a*, *b*). Supplementary hauls (Table 2) were taken with other trawls of various dimensions.

The fish were mostly caught off Mountstuart House on the east side of the Isle of Bute at a depth of about 40 m. Before the final adoption of this sampling area, hauls were taken at various places round the Isle of Cumbrae, though prior to and during the spawning time samples were also obtained from the deeper water between Bute and Cumbrae. During the 1955 spawning season hauls were also taken at other places in the Clyde Sea area (see Table 2 and p. 348). As will be shown later, there is a segregation of mature and immature fish, and the more scattered sampling areas at spawning time were chosen to avoid any bias that this might produce.

The fish were examined fresh in the laboratory. For the purposes of this study the following information was obtained for each individual fish: overall length, sex, stage of maturity, weight of the gonads, and gutted weight; the otoliths were kept for age determination. The overall length was measured from the tip of the lower jaw to the end of the longest caudal fin ray to the nearest 0·5 cm. Later, however, the measurements were grouped into centimetre groups which ranged from $x-0{\cdot}25$ to $x+0{\cdot}75$ cm; these were, however, all classed as x cm rather than $x{\cdot}25$ cm for simplicity in computation. Each fish was assigned to a 'gonad stage' according to an arbitrary classification:

Stage 1. Fish with small translucent, clear or slightly pinkish gonads. It was thought that these fish would not spawn during the next season.

Stage 2. Fish with larger, opaque ovaries in which the eggs were usually visible and opaque. These were thought to be ripening fish that would spawn during the next season.

Stage 3. Fish with at least some translucent eggs in the ovary which had a 'plum-pudding' appearance. These fish were thought to be very close to the time of spawning and included those that were actually ripe and running.

Stage 4. Spent fish with large, blood-shot, empty and flaccid ovaries.

Later, two further stages were introduced, stage 4_1 to include immature fish that had apparently spawned during the previous season, and stage 4_2 for maturing fish which appeared to be recovering from the spent condition. This classification was as far as possible based on what the future breeding condition was supposed to be. It has the advantages of simplicity and of not requiring later regrouping of stages to provide sufficient numbers for analysis.

The classification of the maturity of the males was difficult and no clear stages in gonad development could be found. In any case the numbers caught were so small that analysis of different stages was not practicable.

The gonad weight, and the gutted weight without gonads (in this paper called simply 'weight') were taken to the nearest 0·5 g.

The treatment of the otoliths in age determination has already been described (T. B. Bagenal, 1955*a*).

During the spawning period 20 min hauls were taken with a coarse plankton net at the surface to collect Long Rough Dab eggs. These hauls, taken regularly twice a week in Fairlie Channel, were supplemented by others from elsewhere. Later, hauls were taken for larval and post-larval stages and the young bottom-living fish were caught with a small beam trawl with $\frac{1}{4}$ in. bar cotton netting.

THE LENGTH–WEIGHT RELATION AND CONDITION

Before analysing the data on the breeding and fecundity of the Long Rough Dab it was necessary to consider the condition of the fish; many breeding phenomena might be associated with the well-being of the particular individuals. While there are a number of ways by which the condition of a Long Rough Dab might be measured, the obvious choice with the data available was the weight of the fish for a given length. It seems a reasonable assumption that the heavier fish of a given length are in better general health. In the process of arriving at the relative condition of the Long Rough Dabs, the length–weight relation was analysed, and since this relation is of interest in itself, it will be considered here.

THE LENGTH–WEIGHT RELATION

The details of the analyses of the relation of length to weight for the fish are given in Appendix 1. It is shown that for most groups of fish the weight increases at a rate slightly greater than the cube of the length (Fig. 1). This power was not found to differ between different age-groups or between the males, and immature, maturing and spent females. However, significant differences were found between the powers for immature females caught on different dates. The value of the power ($=b$, the coefficient of the regression of the logarithms of weight on length) is shown in Fig. 2 and appears to vary in an annual cycle. The significance of this would seem to be that if all the fish are gaining in weight and the power is increasing, the larger fish must be putting on weight faster than the small ones, whereas if the power is decreasing the smaller fish must be gaining faster. This aspect of the length–weight relation will be discussed in connexion with the condition of the Long Rough Dabs.

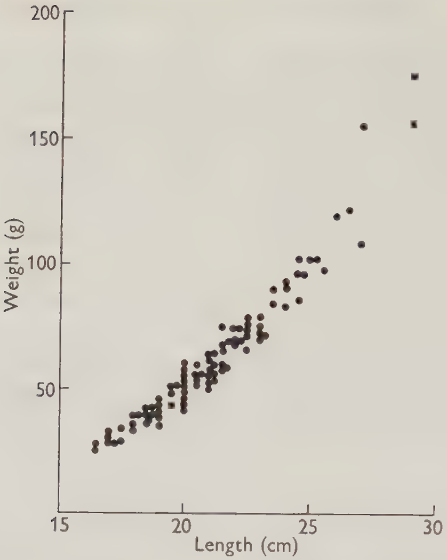

Fig. 1. Scatter diagram of length and weight of maturing (stage 2) female Long Rough Dabs caught on 2 March 1954.

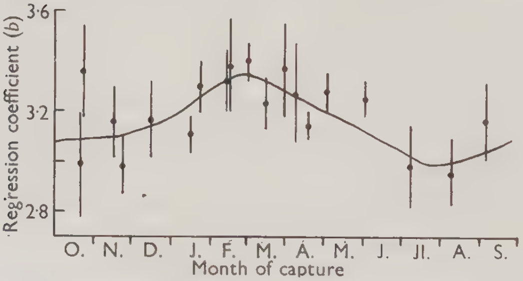

Fig. 2. The regression coefficients (*b*), with their 95 % fiducial limits (vertical lines), for the regressions of log weight on log length of immature female Long Rough Dabs.

THE SEASONAL CYCLE IN CONDITION

The measure of condition has been calculated as the expected weight of a 20 cm Long Rough Dab, derived from analysis of the length–weight relation. The condition of the males, immature, maturing and spent females was found to differ from month to month, and the results are illustrated diagrammatically in Fig. 3. The age-groups within these classes were pooled because on only one of the four dates tested was there a possibly significant difference in their condition.

The data are most complete for the immature female fish. It can be seen that these reach their peak of condition in November–December, and then they lose condition until late April after which there is a recovery. In Fig. 4 are shown the cycles in condition and regression coefficients together. This figure suggests that at first the smaller fish both lose and gain condition faster than larger ones, though later this is reversed and the large fish tend to catch up.

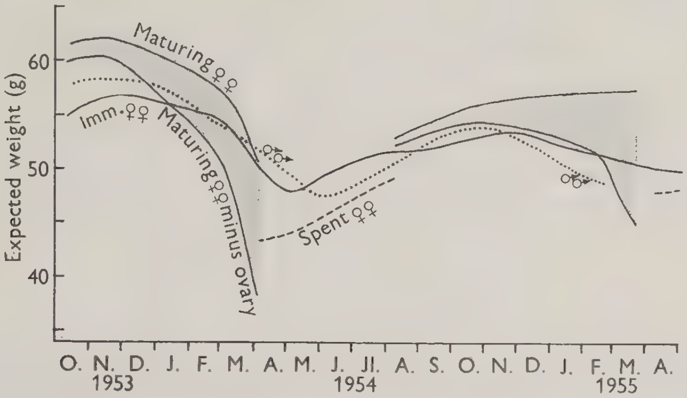

Fig. 3. Diagram of the expected weights of 20 cm male, immature, maturing and spent female Long Rough Dabs from October 1953 to April 1955. The line for spent females continues low in July and August 1954 because those fish that were obviously recovering to spawn again were classified as maturing females.

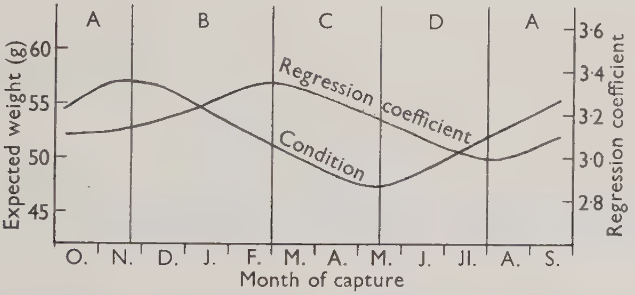

Fig. 4. Diagram of the cycles of regression coefficients (*b*) and condition of immature female Long Rough Dabs, based on Figs. 2 and 3. Period A: August–November; all fish gaining in weight, large fish gaining faster than small fish. Period B: November–February; all fish losing weight, small fish losing faster than large ones. Period C: March–May; all fish still losing, large fish now losing faster. Period D: May–July; all fish gaining in weight again; the small fish gaining faster than the large fish.

The condition of the maturing females is also shown in Fig. 3. It will be realized that any change in condition might be masked by changes in the gonads. The condition has therefore been calculated, both from the total weight including the gonads and from the weights excluding gonads. It can be seen that the total condition (based on weight including gonads) is greater than that of the immature fish, but during March those fish which spawn late lose condition very rapidly and that even earlier the maintenance of the total condition is due to the developing gonads at the expense of the condition of the rest of the fish. The condition of the spent fish is considerably lower than any other group but recovery is rapid, and by August is at approximately the same level as the other groups. The regression coefficients (Table 17) suggest that during autumn and winter the condition of the maturing fish changes similarly in all size-groups until the fall in March when the larger fish are affected most. With spent fish the larger specimens recover faster until May when the recovery of the smaller fish is most rapid.

The data for the male Long Rough Dabs are unfortunately rather meagre, though the cycle in condition appears to be similar to the other groups, being high during autumn and winter and falling to the lowest level in May.

GENERAL BREEDING BIOLOGY

THE SEX RATIO

It is apparent from Table 1 that there are many fewer males than females in the catches. This might be due to the smaller size of the males, which would be able to escape through the net more easily (T. B. Bagenal, 1955*b*), but even for comparable size-groups the males are less numerous than the females, and as the females grow faster, one would expect to find more males in the small size-groups if equal numbers are produced and survive. It seems very unlikely that such unequal numbers of the two sexes would be produced at spawning, and the apparent difference in later life may be due either to differences in the population, or in the samples. The former would include such factors as different habitat preferences, while different behaviour towards an approaching trawl, or different reactions once caught, would be included in the latter. It should be recorded that in no sample ever taken in the Clyde area has a population predominantly male been caught, and the reason for the anomalous sex ratio has not yet been found.

THE RELATION OF MATURITY AND AGE, LENGTH AND WEIGHT

The data are given in Tables 3–7, and are based on the fish caught during the 6 months, October to March, prior to spawning (in 1954 there was no December sample). The two years 1953–54 and 1954–55 are treated separately, though the combined figures are also given, and the data are illustrated in Fig. 5 A–C.

The relation of maturity and age is given in Table 3 and Fig. 5 C. The results for the two years are quite comparable. It will be noticed that there is no definite age at which the fish become mature, for although the percentage mature increases with age, immature specimens of all but the oldest fish have been found. Data concerning the relation of maturity and length are given in Table 4 and Fig. 5 B. The agreement between the two years is again good, and, as with age, there is no length above which all fish are mature. The single immature individual in the 32 cm size-group (6+ years of age) is probably of no significance. The data are re-arranged in Table 5 and Fig. 5A to illustrate the relation of maturity and weight. The same relationship can be seen, though in this case the agreement between the two years is not apparently so good.

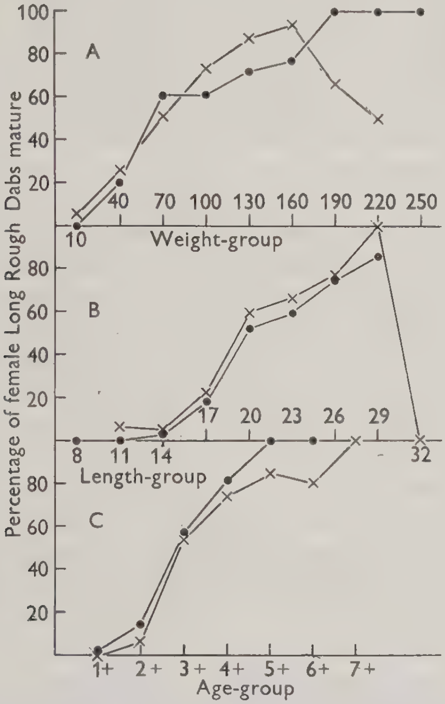

Fig. 5. The relation of percentage maturity to weight (A), length (B) and age (C) of female Long Rough Dabs, based on the 6 months prior to spawning, ●—●, 1953–54; ×—×, 1954–55.

Within a given age-group there is considerable variability in the length and weight of the fish. Tables 6 and 7 give the distribution of mature and immature fish in length- and weight-groups of Long Rough Dabs of the same age. Only in the 3+ and 4+ age-groups were there sufficient fish, well enough divided between mature and immature, to give adequate numbers in each class for comparisons to be possible. The means given in Tables 6 and 7 are based on the original data. An analysis of the percentages of mature fish in subgroups of the same age and length, and of the same age and weight are given in Appendix 2. The results of these tests show that the variability between the percentages for different ages with the same length is significantly greater than that between pairs of groups of fish of the same age and length caught in 1954 and in 1955, though the variability between those for length-groups of the same age is not. This suggests that maturity is more closely linked with the age of the fish than its length. Thompson (1915), working on the Halibut, similarly found 'that the maturity is entirely dependent on age, not on size...'.

A further comparison of the relation of maturity, length and weight is made when we consider the condition of the mature and immature fish. A discussion of the relative condition of Long Rough Dabs has already been given, and it was seen (Fig. 3) that the immature females increase in condition during the summer and autumn and decline again from November to May. The mature fish, though in comparable condition in August, put on total weight faster than the immature ones. This is to a large extent an increase in gonad weight at the expense of the rest of the animal which is relatively in poorer condition than that of the immature specimens. The fish are in their lowest condition just prior to spawning and when newly spent. It also appears that those fish which spawn late in the season lose the most weight. This suggests that there is some survival value to the fish to spawn early, but the maintained regularity of spawning date suggests that there must be some compensating factor. This might well be an increased survival value to the offspring by being spawned late when the water temperatures are more suitable for rapid growth.

It has been suggested that Long Rough Dabs do not necessarily spawn every year, having once attained a certain age or size. It would be of great interest to know when and how it is determined whether the fish will spawn during the next season. The data in the present study are not sufficiently detailed to give any answer, and it is only possible to note that stage 2 fish were not identified before July and only in August were there sufficient numbers for analysis. Those spent fish which had gonads developing for the next season were in significantly better condition than those classed with the immature fish.

THE SEASONAL CYCLE IN GONAD WEIGHT

It was clear from a preliminary examination of the data that the weight of the gonad increased with the size of the fish, but the effect of the age on gonad weight was not apparent. The statistical analysis of the data is given in Appendix 3, where it is shown that for fish of a given length or weight there is no difference in the gonad weights of the different age-groups. After pooling the ages, the expected gonad weights for mature female Long Rough Dabs of 69·98 g were calculated for the months prior to spawning and these, shown in Fig. 6, illustrate the rate of development of the maturing ovaries.

THE SPAWNING TIME

In Table 1 can be found the dates of capture and the numbers of Long Rough Dabs of each maturity stage that were caught. The percentages of immature, ripe and spent of the total mature fish are given in Fig. 7, which also includes a graph of the numbers of Long Rough Dab eggs in 20 min tow-net hauls off Keppel Pier. From this figure it can be seen that the spawning times in 1954 and 1955 were very similar, and lasted from the beginning of March until the end of the second week in April, and half the fish had spawned by 20–22 March. It is at this time too that the fish are in the poorest condition, though the lowest level reached by the males is later than that of the mature females.

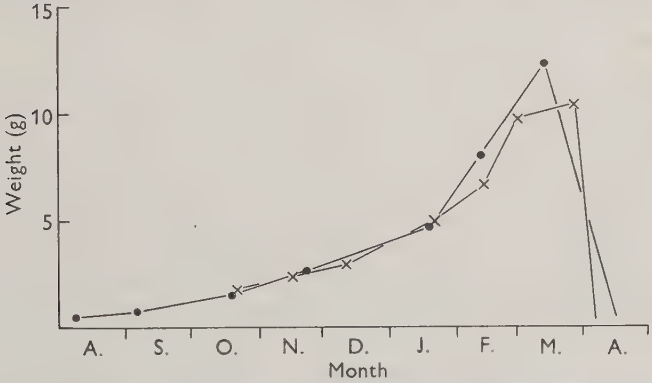

Fig. 6. The expected gonad weight of a female Long Rough Dab 69·98 g in weight, from October 1953 to March 1954 (×—×), and from August 1954 to March 1955 (●—●).

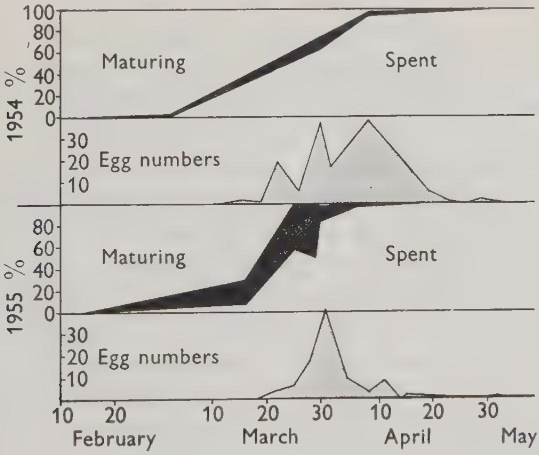

Fig. 7. The percentage of maturing, ripe (solid black) and spent female Long Rough Dabs through the 1954 and 1955 breeding seasons. The numbers of Long Rough Dab eggs in 20 min plankton hauls are also illustrated (stippled frequency diagram).

The egg collections support these conclusions very well. It appears from the small percentage of stage 3 fish caught on each occasion (never more than 50%) that this stage is of short duration for each individual fish, as was found by Fulton (1890). In this respect it is of interest that only one fish (caught on 29 March 1955) was actually fully ripe with running eggs. A comparison with previous studies in other regions, based on egg surveys or the maturity stage of the adult, is given in Table 8.

The relation of spawning time and age is shown by the data in Table 9, which suggests that the older fish spawn first. It has also been found that the larger fish spawn first; the mean length of the spent fish on 30 March 1954 was 22·7 cm compared with 19·5 cm for those that had not yet spawned. Unfortunately the data are not sufficient to allow further subdivision and to test the relation of spawning time and length within each age-group.

THE SPAWNING PLACE AND DEPTH

The main sampling area was on the west side of the channel between the islands of Bute and Cumbrae at a depth of about 40 m off Mountstuart House. During the spawning season hauls were also taken in the deeper parts of the channel in 60–70 m. Comparisons of the catches are given in Table 10. These data, in conjunction with the details of the supplementary hauls in Table 2, show that the mature and spent dabs are found most commonly at depths of between 60 and 90 m, and these results confirm those of Fulton (1890) that the fish spawn in deep water. The absence of Long Rough Dabs in the catch on 6 April 1955 in 150 m confirms the general impression, gained over a number of years, that these fish are very rare in the deepest water of the Clyde area.

THE GROWTH AND DEVELOPMENT OF THE EGGS AND LARVAE

The eggs of the Long Rough Dab were found in plankton catches obtained from 20 min hauls in Fairlie Channel from the middle of March to the beginning of May. Although the same kind of net was always used (mouth diameter 45 cm, 26 mesh to linear inch) the hauls cannot be considered strictly quantitative owing to a number of factors. The catches, however, do show the approximate duration of Long Rough Dab eggs in the plankton, and when the greatest numbers were present (Fig. 7).

No attempt was made to catch the larval Long Rough Dabs before the yolk-sac was absorbed. They were not seen in the plankton hauls, but were probably overlooked. However, post-larvae were found and the details of the catches are given in Table 11; the rate of growth of these larvae is illustrated in Fig. 8. It was found that metamorphosis commenced when the larvae were about 15 mm long and that they took to the bottom as fully metamorphosed fish at about 25 mm in length. There was considerable individual variation about these average sizes.

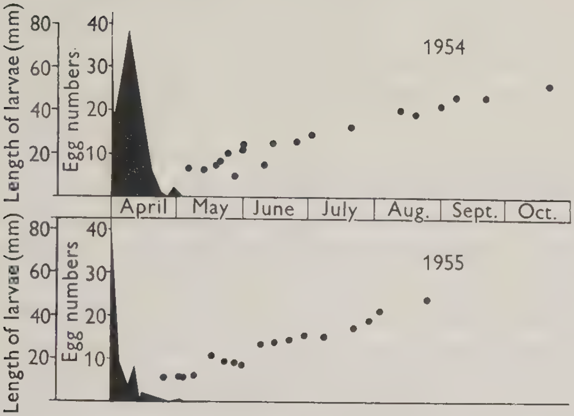

Fig. 8. The lengths of post-larval Long Rough Dabs during the summers of 1954 and 1955. The decline in egg numbers in the plankton hauls is shown in solid black for each year.

FECUNDITY

During the study of the breeding biology, fecundity estimations were made for 119 of the stage 2 Long Rough Dabs. The fish were mainly from two samples, the first consisting of the 25 fish obtained on 15 February 1954, and the second of the 91 fish on 2 March 1954 (see Table 1). On these dates all the maturing female Long Rough Dabs that were caught were examined; and they may be considered as random samples from the population; the stage 3 fish on 2 March 1954 was not included since some of its eggs may have already been shed. The remaining three fish were obtained on 15 and 30 March, and were selected because they represented the smallest size-groups of mature females and were supposed to supplement the two random samples. After the usual treatment of the fish (see p. 340), the gonads were preserved. The full details for each individual were kept separate, and these are given in Table 30.

The method of preservation and subsequent treatment of the ovaries followed that used by Simpson (1951) for plaice. The ovaries were preserved and stored in Gilson's fluid modified by doubling the amount of acetic acid. This not only preserved the eggs but also helped to liberate them by breaking down the ovarian tissue. The process was aided by repeated, though spasmodic, shaking of the bottles in which the ovaries were preserved. Before the eggs were to be counted the ovaries were teased apart and the ovarian tissue removed and any adhering eggs were returned to the bottle. Next the eggs were washed by successive shaking in Gilson's fluid and decanting the supernatant liquid, though if the eggs were to be counted immediately water was used and this was not found to affect the eggs. The decanting removed not only remaining pieces of ovarian tissue, but also those minute eggs which would be laid in later years and have caused difficulties in the past (Fulton, 1891; Mitchell, 1913). Lastly, the eggs were examined in a Petri dish and any remaining tissue removed, and clumps of eggs were broken up.

Estimations of the egg numbers in ovaries of each fish were obtained from the means of four subsamples of 1/200 each. The eggs were placed in a cylindrical museum jar and water was added until the volume was 200 times the capacity of the subsampler, which consisted of a small specimen tube attached to a glass rod. For most of the counts the tube held 13 ml., but in the earlier subsamples one of 12 ml. was used. The eggs were stirred vigorously with a non-rotary action until apparently randomly distributed. The subsampler was quickly filled, and the contents washed into a Petri dish. This process was repeated four times, and each of the subsamples counted.

In order to test the accuracy of the method 40 counts were made on the same batch of eggs and these are given in Table 12. The counts were made in series of four and the subsamples were returned to the jar after each series. The standard deviation of forty observations was 97·308 corresponding to a coefficient of variation of 10·05%. For the means of four the standard deviation is 48·654 ($97{\cdot}308/\sqrt{4}$) corresponding to a coefficient of variation of 5·03%.

This may be compared with the method given by Simpson who used a 1 ml. stempel pipette from a 150 ml. whirling flask and got a standard deviation for forty observations of 35·393 or $C = 9{\cdot}57\%$ and for means of 4 of 4·78%. The coefficient of variation based on his actual means was 3·64%, and in the present work 7·05%. This indicates that Simpson's actual means were less variable than might be expected by chance from the separate counts, while those given in Table 12 are more variable.

The analyses of the relations of fecundity to age, length, weight and gonad weight are given in Appendix 4.

RESULTS

The data given in Appendix 4 show that the fecundity of the female Long Rough Dabs varies from 25,000 eggs for a fish of *ca.* 15 cm and 20 g to 250,000 for one of *ca.* 30 cm and 200 g. The statistical analysis of the data shows that there is very great variability in the fecundity, and that while the general level can be related to the age, length, weight and gonad weight of the fish, there is still a large amount of variability after these factors have been taken into account. The analyses show that for estimating the fecundity, weight and gonad weight used together are the most accurate. However, since length is so much easier and quicker to measure, if time is limited and the fish are not, it might be more suitable to predict fecundity from a larger sample using only the length data. The variability is shown diagrammatically as scatter diagrams of fecundity and length, weight and gonad weight in Figs. 9–11. The fecundity has been found to increase at a rate slightly above

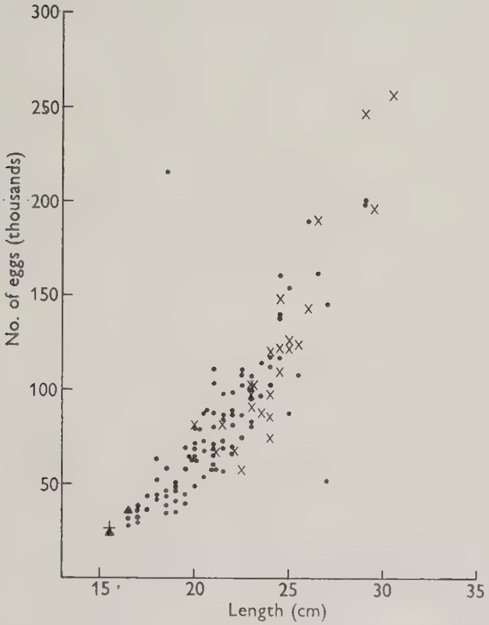

Fig. 9. Scatter diagram showing the relation of fecundity and length of female Long Rough Dabs. ×, 15 February 1954; ●, 2 March 1954; +, 15 March 1954; ▲, 30 March 1954.

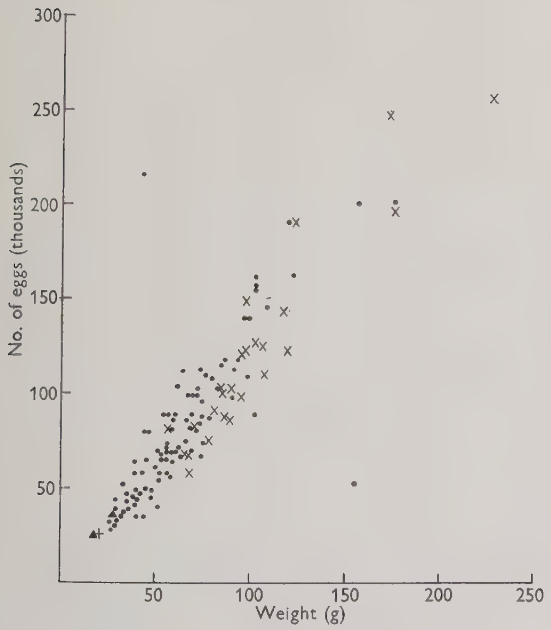

Fig. 10

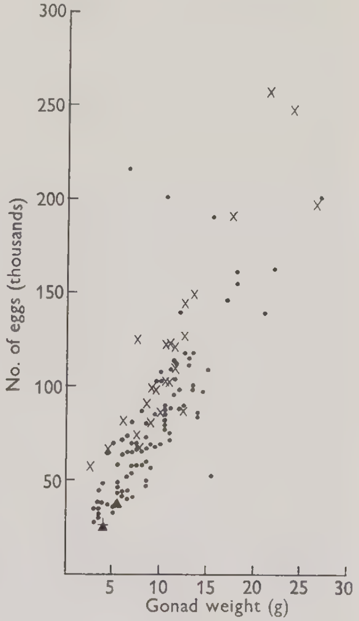

Fig. 11

Fig. 10. Scatter diagram showing the relation of fecundity and weight of female Long Rough Dabs. Symbols as in Fig. 9.

Fig. 11. Scatter diagram showing the relation of fecundity and gonad weight of female Long Rough Dabs. Symbols as in Fig. 9.

the cube of the length. The relation of fecundity and age is, in so far as there is a relationship at all, almost linear, but since there are so few age classes it is obvious that the variability will be large. The relation of fecundity to weight is also probably linear, which is to be expected since the relation of weight to length is similar to that of fecundity to length. The relation of fecundity to gonad weight is shown in Fig. 11 and is non-linear. Fulton's (1891) five fecundity estimates are a little lower than those given here for fish of a comparable length, but the regression of log fecundity on log length is not linear for the data he gives, which suggests that the estimates may not be reliable.

Mitchell (1913) observed three sizes of eggs in the ovaries she examined. If those estimates of fish evidently in the maturity stage 3 condition, and also the minute eggs to be spawned in later years, are ignored, the estimates do not differ significantly from those given here.

Milinsky (1944) gives egg numbers for three fish of 47, 48 and 49 cm in length. Long Rough Dabs of this size are never found in the Clyde area, but the fecundity he gives is significantly lower than would be expected from the regression equation given in this paper. A Clyde Long Rough Dab of about 32 cm would be expected to have a fecundity comparable to that given by Milinsky for his fish.

DISCUSSION

Simpson's (1951) interest in the fecundity of the Plaice, *Pleuronectes platessa*, was primarily directed to obtaining reliable data for population estimation from planktonic egg surveys (Buchanan-Wollaston, 1923). Other interest centres round the problem of determining the density-dependent factors affecting the population size. It has been argued by Lack (1954) that since fecundity is associated with the size rather than the age of the parent, and also that since fish tend to grow faster at low population densities than in overcrowded conditions, where food is supposed to limit growth, the population density in natural conditions is controlled by the available food. The analysis of the fecundity data given in this paper confirm the closer association of fecundity with size than with age. However, it is also shown that in Long Rough Dabs the percentage of mature fish in the population is associated with the age structure and not the size-frequency distribution. Thus with a moderate increase in the predation of the older and larger fish the number in the breeding stock is diminished, though each individual produces more eggs.

The problem of natural death in fishes was considered by Orton (1929) who, using Russell's (1914) data for Haddock, *Gadus aeglefinus*, suggested that death might be brought about by what could be called 'over reproduction'. Russell's evidence showed that the gonad weight to total weight ratio increases with the size of the fish, and Orton suggested that this tendency would produce a size limit above which the fish could not survive. In the breeding data on the Long Rough Dab analysed in this paper it has been shown that relation of gonad weight to weight is linear and no trend was found in the relation with increasing age (p. 373). However, another aspect of the same problem is seen when we consider the fecundity. Milinsky's very large dabs did not produce the egg numbers expected from their size. Furthermore, from a consideration of the regression of log fecundity on log gonad weight (Fig. 11), it appears that the number of eggs in the gonad does not increase in proportion to the weight. This result was unexpected since the larger gonads will have a proportionally smaller surface area and so, not only should carry less surface moisture when they are weighed, but also less ovarian tissue should be found surrounding the eggs in the larger gonads. We can only suppose that the heavier gonads produce fewer eggs per gram than do the lighter ones, so the eggs are presumably larger and heavier. In future work an analysis of the eggs is needed to show how the fecundity is related to the food reserves in the eggs themselves, and so perhaps to their viability. To a large extent in fish, significance of the fecundity is obscured by the enormous numbers of eggs laid and its dependence on the size of the parent, but the significance of clutch-size in birds and litter-size in mammals have been discussed by Lack (1947, 1948*a*, *b*). It would appear that, as in these other groups, if other factors are equal the eggs from the more fecund parents would have greater chances of survival than those from the less fecund. Unless there were some compensating factor, a mutation tending to produce greater fecundity would pass through the whole population. It has, in the past (Simpson, 1951), been suggested that the factors governing the fecundity of individual fish might be: (*a*) the condition of the fish when the germinal epithelium is laid down during the first year of life; and (*b*) the condition of the fish either when the eggs to be laid each year are separated from the mass of developing ova, or when the new primary oocytes are being formed each year. The condition of the fish at these critical times is expected to be closely associated with the food supply and the temperature of the environment. These environmental factors may indeed be expected to influence the fecundity of individual fish within limits set by their hereditary characteristics. A mutation, tending to increase the general level of fecundity, would still be expected to spread through the entire population were it not for some compensating factors, which reduce the chances of survival of the entire progeny of a particularly fecund individual. Such compensating factors may act on the parent or the eggs. After spawning the female fish are in the poorest condition reached during the whole year. If fecundity and post-spawning condition were inversely correlated and the condition of the fish critical to their survival, the more fecund fish might not live to spawn again or only to spawn two seasons hence. With the data available there is no evidence of the more fecund fish being in poorer condition (Appendix 4, p. 375).

It is clear from the earlier section that some fish do not spawn every year having once reached sexual maturity. It is, however, difficult to say if this is associated with greater fecundity in the previous season.

Factors associated with the survival of the progeny would include the possibility of the eggs being smaller, and containing less food reserves.

While it is not possible at this present stage to suggest which of these factors is the most likely to control the fecundity of a given species of fish, these are the lines on which future work might profitably be directed.

SUMMARY

Details are given of the breeding biology, including the fecundity, of the Long Rough Dab deduced from samples taken in the Clyde Sea area from October 1953 to April 1955. Where possible breeding is related to the condition of the fish; the index of condition is based on the length–weight relation.

The weight of Long Rough Dabs of each sex and stage in maturity increases as a power, slightly greater than the cube, of the length. An annual cycle of fluctuations of this power is seen in the immature females, and indicates how changes in condition at different seasons affect the different size-groups. The measure of condition is taken as the expected weight of a 20 cm Long Rough Dab, and the changes in this weight are followed through the sampling period for males and for immature, maturing and spent females. All groups tend to be in best condition during November and December followed by a decline to the spawning time, in March and April, after which there is a slow recovery.

The scarcity of males in the samples cannot be satisfactorily explained. There is no definite age, length or weight above which all the female fish become mature, but the percentage of mature fish is more closely linked to the age structure than to the size-distribution. The condition of the mature and immature fish is compared; mature females are initially in better condition, and as the breeding season approaches this is maintained by the gonads developing at the expense of the flesh. The relations of the gonad weight to length and to weight appear to be similar in all age-groups, and the pre-spawning increase in gonad weight is discussed.

The spawning season was determined from the disappearance of mature fish from the samples and their replacement by spent females, and by the appearance of eggs in the plankton; it extends from the beginning of March to the middle of April. The older and larger fish have been found to spawn first; spawning takes place predominantly at depths of 60–90 m. The eggs remain in the plankton in quantity for 3–4 weeks. The larvae metamorphose at about 15 mm, take to the bottom at approximately 25 mm and for the first 6 months grow at about 10 mm per month.

The fecundity was estimated from four subsamples of 1/200 of the eggs from each of 119 fish, mostly caught on two dates. The egg numbers varied

from 25,000 for a fish of 15 cm to over 250,000 for a 30 cm fish. The data were subjected to multiple regression analyses and it is seen that the fecundity is most accurately estimated from the weight and gonad weight together, and the addition of length to these measurements does not increase the efficiency significantly. The fecundity was found to be related to weight linearly, to the length at a power greater than the cube and to the gonad weight at a power less than unity. The significance of these relations and a discussion of the wider implications of the paper is given.

REFERENCES

BAGENAL, M., 1955. A note on the relations of certain parameters following a logarithmic transformation. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 289–96.

BAGENAL, T. B., 1955*a*. The growth rate of the Long Rough Dab *Hippoglossoides platessoides* (Fabr.). *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 297–311.

—— 1955*b*). The growth rate of the Long Rough Dab *Hippoglossoides platessoides* (Fabr.)—a correction. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 643–7.

BAL, D. V. 1943. A study of fish eggs and larvae from Manx waters. I. Observations on the fish eggs. *J. Univ. Bombay*, N.S. 11 B (5), pp. 54–90.

BIGELOW, H. B. & SCHROEDER, W. C., 1953. Fishes of the Gulf of Maine. *Fish. Bull., U.S.*, Vol. 53, No. 74, 577 pp.

BUCHANAN-WOLLASTON, H. J., 1923. The spawning of plaice in the southern part of the North Sea in 1913–14. *Fish. Invest., Lond.*, Ser. 2, Vol. 5, No. 2, 36 pp.

COCHRAN, W. G., 1947. Some consequences when the assumptions for the analysis of variance are not satisfied. *Biometrics*, Vol. 3, pp. 22–38.

ESSIPOW, W. J. & SLASTNIKOW, G., 1932. Zur Biologie des Kambalajorsch (*Drepanopsetta platessoides* O. Fabr.) im Barentsmeer. *Wiss. Fischereiuntersuchungen am Murman* (*Barents Sea*), pp. 191–8. (In Russian: German summary.)

EWART, J. C. & FULTON, T. W., 1889. Report on the spawning of the British marine food-fishes. *Rep. Fish. Bd Scot.*, Vol. 7, pp. 186–221.

FULTON, T. W., 1890. The spawning and spawning place of marine food-fishes. *Rep. Fish. Bd Scot.*, 8 (1889), Part III, pp. 257–69.

—— 1891. On the comparative fecundity of sea fish. *Rep. Fish. Bd Scot.*, 9 (1890), Part III, pp. 243–68.

—— 1904. The rate of growth of fishes. *Rep. Fish. Bd Scot.*, 22 (1903), Part III, pp. 141–241.

GÜNTHER, A., 1888. Report on the fishes obtained by Mr J. Murray in deep water on the north-west coast of Scotland between April 1887 and March 1888. *Proc. roy. Soc. Edinb.*, Vol. 25, pp. 205–20.

HUNTSMAN, A. G., 1918. Histories of new food fishes. 1. The Canadian plaice. *Bull. biol. Bd Can.*, No. 1, 32 pp.

KRÜGER, K. VON, 1942. Erneutes Auftreten der Scharbzunge, *Drepanopsetta platessoides* Fabr. in der westlichen Ostsee. *Kieler Meeresforsch.*, Bd. 4, pp. 18–37.

LACK, D., 1947. The significance of clutch size. *Ibis*, Vol. 89, pp. 302–52.

—— 1948*a*. The significance of clutch size. *Ibis*, Vol. 90, pp. 25–45.

—— 1948*b*. The significance of litter size. *J. Anim. Ecol.*, Vol. 17, pp. 45–50.

—— 1954. *The Natural Regulation of Animal Numbers*. 343 pp. Oxford.

LECREN, E. D., 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the Perch (*Perca fluviatilis*). *J. Anim. Ecol.*, Vol. 20, pp. 201–19.

MATHER, K., 1946. *Statistical Analysis in Biology*. Second edition. London: Methuen.

MILINSKY, G. I., 1944. On the biology and fisheries of the long rough dab of the Barents Sea. *Trans. Knipovich polyar. sci. Inst.*, Vol. 8, pp. 388–415. (In Russian: English summary.)

MITCHELL, A. M., 1913. On the egg production of certain fishes. *Rep. N. Sea Fish. Invest. Comm.*, 5th Report (Northern Area), pp. 191–204.

NORMAN, J. R., 1934. *A Systematic Monograph of the Flatfishes (Heterostomata)*. Vol. 1. London.

ORTON, J. H., 1929. Reproduction and death in invertebrates and fish. *Nature, Lond.*, Vol. 123, pp. 14–15.

OTTERSTRØM, A., 1906. Eggs and young of fishes in the Danish waters. *Rep. Danish biol. Sta.*, Vol. 13, 85 pp.

PEARSON, E. S. & HARTLEY, H. O., 1954. *Biometrika Tables for Statisticians*, Vol. 1. Cambridge University Press.

PERTSEVA, T. A., 1939. Spawning, eggs and fry of fish in the Motovski Bay. *Trans. Inst. mar. Fish. USSR.*, Vol. 4, pp. 417–70. (In Russian: English summary and table headings.)

RASS, T. S., 1936. Spawning, eggs and fry of the food-fishes of the Barents Sea. Breeding and development of fish in the subarctic. *Int. Rev. Hydrobiol.*, Vol. 33, pp. 250–70.

RUSSELL, E. S., 1914. Report on the market measurements in relation to the English haddock fishery during the years 1909–1911. *Fish. Invest., Lond.*, Ser. 2, Vol. 1, No. 1, 134 pp.

SAEMUNDSSON, B., 1908. Oversigt over Islands Fiske med oplysninger om deres forekomst vigtigste biologiske forhold og okonomiske betydning. *Skr. Komm. Havunders, Kbh.*, No. 5, 140 pp.

—— 1925. Fiskirannsoknir 1923–1924. *Andvari, Reykjavik*, pp. 33–71.

—— 1927. Synopsis of the fishes of Iceland. *Rit Vísind. Isl.*, Vol. 2, 66 pp.

—— 1949. Marine Pisces. *Zoology of Iceland*, Vol. 4, Pt. 72, 150 pp.

SIMPSON, A. C., 1951. The fecundity of the plaice. *Fish. Invest., Lond.*, Ser. 2, Vol. 17, No. 5, 27 pp.

SNEDECOR, G. W., 1946. *Statistical Methods*. Fourth edition. Iowa State College Press.

THOMPSON, W. F., 1915. A preliminary report on the life-history of the halibut. *Rep. Comm. Fish. B.C.*, 1914, pp. 76–120.

WILLIAMSON, H. C., 1899. On the pelagic fish-eggs and larvae of Loch Fyne. *Rep. Fish. Bd Scot.*, 17 (1898), Part III, pp. 79–131.

TABLE 1. DETAILS OF CATCHES

Date 1953	Males	Females Stage 1	Stage 2	Stage 3	Stage 4	Total females	Grand total
23. x.	7	30	35	—	—	65	72
19. xi.	10	26	30	—	—	56	66
15. xii.	5	27	21	—	—	48	53
1954							
21. i. 26. i.	5	46	40	—	—	86	91
15. ii.	—	24	25	—	—	49	49
2. iii.	10	176	91	1	—	268	278
30. iii.	—	68	9	4	21	102	102
8. iv.	—	67	3	3	74	147	147
3. v.	5	74	—	—	43	117	122
2. vi.	30	111	—	1	64	176	206
8. vii.	16	57	5	—	17	79	95
9. viii.	37	46	61	—	9	116	153
7. ix.	12	31	36	—	1	68	80
21. x.	12	31	22	—	—	53	65
25. xi.	8	76	86	—	1	163	171
1955							
20. i.	3	69	41	—	—	110	113
14. ii.	5	35	36	—	—	71	76
16. iii.	2	51	12	4	1	68	70
18. iv.	2	73	—	—	36	109	111

TABLE 2. DETAILS OF CATCHES IN SUPPLEMENTARY HAULS

Date 1955	Position	Depth (m)	Males	Females Stage 1	Stage 2	Stage 3	Stage 4	Total females	Grand total
14. ii.	Bute Channel	60–70	1	18	15	—	—	33	34
	Mountstuart	40–50	—	27	12	—	—	39	39
25. iii.	Skelmorlie	35–45	—	7	—	1	1	9	9
	Bute Channel	50–70	—	4	—	4	6	14	14
29. iii.	Bute Channel	60–70	—	—	—	2	1	3	3
	Bute Channel	60–70	—	1	—	—	—	1	1
	Mountstuart	40–50	—	27	—	7	6	40	40
	Mountstuart	40–50	2	35	—	4	6	45	47
30. iii.	Off Lady Isle	40–50	1	61	—	7	33	101	102
	Off Ardrossan	50–70	—	29	1	2	16	48	48
6. iv.	Off Sannox Arran 1 m	150	—	—	—	—	—	—	—
	Sannox Arran 2 m	*ca.* 90	—	54	—	1	13	68	68
	Sannox Arran 2 m	*ca.* 90	—	17	—	—	14	31	31
	S.W. Garroch Head	*ca.* 60	—	27	—	—	11	38	38

TABLE 3. THE RELATION OF MATURITY AND AGE

Age-group	1953–54 Immature	1953–54 Mature	1953–54 % mature	1954–55 Immature	1954–55 Mature	1954–55 % mature	Both years % mature
1+	30	1	3·2	3	—	0	2·9
2+	202	35	14·8	147	10	6·4	11·4
3+	81	109	57·4	83	97	53·9	55·7
4+	16	70	81·4	24	69	74·2	77·7
5+	—	27	100	4	22	84·6	92·5
6+	—	1	100	1	4	80·0	83·3
7+	—	—	—	—	1	100	100
Total	329	243	42·48	262	203	43·66	43·01

TABLE 4. THE RELATION OF MATURITY AND LENGTH

Length-group	1953–54 Immature	1953–54 Mature	1953–54 % mature	1954–55 Immature	1954–55 Mature	1954–55 % mature	Both years % mature
8	5	—	0	—	—	—	0
11	16	—	0	13	1	7·1	3·3
14	40	1	2·4	95	5	5·0	4·3
17	119	25	17·4	59	17	22·4	19·1
20	80	88	52·4	49	73	59·8	55·5
23	55	82	59·9	34	68	66·7	62·8
26	12	35	74·5	11	38	77·6	76·0
29	2	12	85·7	—	1	100	86·7
32	—	—	—	1	—	0	0
Total	329	243	—	262	203	—	—

TABLE 5. THE RELATION OF MATURITY AND WEIGHT

Weight-group	1953–54 Immature	1953–54 Mature	1953–54 % mature	1954–55 Immature	1954–55 Mature	1954–55 % mature	Both years % mature
10	61	—	0	101	6	5·6	3·6
40	157	41	20·7	83	29	25·9	22·6
70	58	91	61·1	46	48	51·1	57·2
100	40	63	61·2	24	67	73·6	67·0
130	10	26	72·2	5	35	87·5	80·3
160	3	10	76·9	1	15	93·8	86·2
190	—	8	100	1	2	66·6	90·9
220	—	3	100	1	1	50·0	80·0
250	—	1	100	—	—	—	100
Total	329	243		262	203		

TABLE 6. THE RELATION OF MATURITY AND LENGTH WITHIN AGES 3+ AND 4+

	1953–54				1954–55			
Age ...	3+		4+		3+		4+	
Length-group	Immature	Mature	Immature	Mature	Immature	Mature	Immature	Mature
14	—	—	—	—	2	—	—	—
17	2	6	—	3	19	13	—	—
20	26	52	2	16	40	60	4	11
23	50	47	3	31	21	23	13	32
26	3	3	9	19	1	1	7	26
29	—	1	2	1	—	—	—	—
Total	81	109	16	70	83	97	24	69
Mean	22·16	21·45	24·69	22·97	19·90	20·35	23·25	23·58

TABLE 7. THE RELATION OF MATURITY AND WEIGHT WITHIN AGES 3+ AND 4+

	1953–54				1954–55			
Age ...	3+		4+		3+		4+	
Weight-group	Immature	Mature	Immature	Mature	Immature	Mature	Immature	Mature
10	—	—	—	—	1	—	—	—
40	7	15	1	5	33	22	—	3
70	35	54	2	20	33	39	11	9
100	36	34	3	25	15	30	7	23
130	3	5	7	16	1	5	4	24
160	—	—	3	2	—	1	1	9
190	—	1	—	1	—	—	1	1
220	—	—	—	1	—	—	—	—
Total	81	109	16	70	83	97	24	69
Mean	82·96	80·46	115·63	99·71	56·87	66·29	89·58	98·99

TABLE 8. THE SPAWNING TIME OF THE LONG ROUGH DAB

Authority	Region	Range of spawning time	Maximum spawning
Bagenal (this paper)	Clyde	Feb.–Apr.	Mar.
Williamson (1899)	Loch Fyne	Mar.–June	Apr.
Günther (1888)	W. Scotland	Feb.–Mar.	Early Mar.
Bal (1943)	Irish Sea	Mar.–Aug.	Apr.
Ewart & Fulton (1889)	Firth of Forth	Dec.–May	Mar.
Ewart & Fulton (1889)	Moray Firth	Jan.–Feb.	—
Fulton (1890)	Moray Firth	—	Mainly Mar.
Otterstrøm (1906)	Kattegat & Baltic	—	Mar. only
Pertseva (1939)	Barents Sea	Mar.–June	End of Apr.
Rass (1936)	Barents Sea	Mar.–June	May
Saemundsson (1949)	Iceland	May–June	—
Huntsman (1918)	Bay of Fundy	Apr.–May	—
Huntsman (1918)	Gulf of St Lawrence	May–June	—
Huntsman (1918)	Newfoundland	July	—

TABLE 9. THE AGE AND MATURITY-STAGE DISTRIBUTION AT SPAWNING

		Maturity stage			
Date	Age	2	3	4	Total
30. iii. 54	3	5	1	1	7
	4	2	3	5	10
	5	1	1	12	14
	6	—	—	3	3
	Total	9	4	21	34
16. iii. 55	3	1	—	—	1
	4	8	3	—	11
	5	3	1	1	5
	Total	12	4	1	17
25. iii. 55	3	—	3	—	3
	4	—	2	5	7
	5	—	—	1	1
	6	—	—	1	1
	Total	—	5	7	12

TABLE 10. COMPARISON OF NUMBERS OF IMMATURE AND MATURE LONG ROUGH DABS IN DEEP AND SHALLOW WATER

<table>
<tr><th></th><th></th><th colspan="4">Depth</th></tr>
<tr><th></th><th></th><th colspan="2">40–50 m</th><th colspan="2">60–70 m</th></tr>
<tr><th>Date</th><th></th><th>Haul III</th><th>Haul IV</th><th>Haul I</th><th>Haul II</th></tr>
<tr><td>14. ii. 55</td><td>Stage 1</td><td>31</td><td>27</td><td>15</td><td>8</td></tr>
<tr><td></td><td>Stage 2</td><td>14</td><td>12</td><td>19</td><td>22</td></tr>
<tr><td>2. iii. 54</td><td>Stage 1</td><td colspan="2">84</td><td colspan="2">92</td></tr>
<tr><td></td><td>Stage 2</td><td colspan="2">18</td><td colspan="2">74</td></tr>
<tr><td></td><td>Stage 3</td><td colspan="2">—</td><td colspan="2">1</td></tr>
<tr><td>8. iv. 54</td><td>Stage 1</td><td colspan="2">55</td><td colspan="2">12</td></tr>
<tr><td></td><td>Stage 2</td><td colspan="2">—</td><td colspan="2">3</td></tr>
<tr><td></td><td>Stage 3</td><td colspan="2">1</td><td colspan="2">2</td></tr>
<tr><td></td><td>Stage 4</td><td colspan="2">11</td><td colspan="2">63</td></tr>
<tr><td></td><td>Total non-breeding</td><td colspan="2">197</td><td colspan="2">127</td></tr>
<tr><td></td><td>Total breeding</td><td colspan="2">56</td><td colspan="2">184</td></tr>
<tr><td></td><td>Total</td><td colspan="2">253</td><td colspan="2">311</td></tr>
</table>

TABLE 11. DETAILS OF CATCHES OF POST-LARVAL LONG ROUGH DABS

1954					1955				
Date	No.	Mean (mm)	S.D.	Gear	Date	No.	Mean (mm)	S.D.	Gear
6. v.	6	13·2	3·2	Plankton net	25. iv.	9	10·8	2·4	Plankton net
13. v.	7	12·7	2·6	Plankton net	2. v.	3	11·3	—	Plankton net
19. v.	86	15·2	3·8	Plankton net	4. v.	64	10·8	2·5	Plankton net
20. v.	16	15·9	4·4	Plankton net	9. v.	254	12·2	2·6	Plankton net
24. v.	1	20	—	Plankton net	17. v.	48	21·2	4·5	Plankton net
27. v.	1	10	—	Plankton net	23. v.	4	18·5	—	Plankton net
31. v.	1	22	—	Plankton net	28. v.	1	18	—	Plankton net
31. v.	69	23·7	1·8	Beam trawl	1. vi.	10	17·2	3·4	Plankton net
10. vi.	28	15·1	4·8	Plankton net	9. vi.	46	26·7	2·4	Beam trawl
14. vi.	26	24·8	2·7	Beam trawl	15. vi.	42	27·8	3·3	Beam trawl
25. vi.	13	26·1	2·8	Beam trawl	22. vi.	44	28·5	2·7	Beam trawl
2. vii.	22	29·0	2·8	Beam trawl	30. vi.	61	30·9	3·4	Beam trawl
20. vii.	56	33·0	3·9	Beam trawl	8. vii.	11	30·5	2·5	Beam trawl
12. viii.	26	40·8	3·5	Beam trawl	22. vii.	31	34·5	4·7	Beam trawl
19. viii.	38	38·6	4·5	Beam trawl	29. vii.	31	37·7	4·7	Beam trawl
31. viii.	33	43·1	4·1	Beam trawl	3. viii.	39	41·8	4·4	Beam trawl
7. ix.	21	47·1	4·9	Beam trawl	25. viii.	49	48·2	5·2	Beam trawl
21. ix.	37	46·8	3·6	Beam trawl					
21. x.	62	53·3	5·2	Beam trawl					

TABLE 12. DETAILS OF REPLICATE COUNTS OF LONG ROUGH DAB EGGS

Series no.	1	2	3	4	5	6	7	8	9	10
Count no. 1	1046	940	1051	917	858	1006	919	1032	1038	902
2	851	1069	1006	995	777	933	1153	1044	1009	1016
3	970	994	881	1037	844	796	931	875	1012	935
4	1114	1183	966	985	808	762	1077	960	978	1048
Mean	995·25	1046·5	976·0	983·5	821·75	874·25	1020·0	977·75	1009·25	975·25

Grand mean of 40 observations = 967·95
S.D. = 97·308
Coefficient of variation = 10·05 %

Mean of 10 means = 967·95
S.D. = 68·264
Coefficient of variation = 7·05 %

TABLE 13

Subgroup	S.S.L.	S.S.W.	S. products L.W.	Regression coefficient b	S.S. due to regression	Residual S.S.	Degrees of freedom
Age 1+	0·003397	0·054392	0·011935	3·513394	0·041932	0·012460	6
2+	0·223974	2·827187	0·764614	3·413852	2·610279	0·216908	124
3+	0·040840	0·535860	0·137443	3·365402	0·462551	0·063309	33
4+	0·008973	0·118283	0·032089	3·576173	0·114756	0·003527	4
					Total	0·296204	167
Total within ages	0·277184	3·535722	0·946081	3·413188	3·229152	0·306570	170
					Difference	0·010366	3
Between ages	0·964671	11·123082	3·274817	3·394750	11·117185	0·005897	2
					Sum	0·312467	172
Total	1·241855	14·658804	4·220898	3·398865	14·346264	0·312540	173
					Difference	0·000073	1

TABLE 14

Line	Source	Sums of squares	Degrees of freedom	Mean square	Variance ratio	Significance
1	Due to total regression	14·346264	1	14·346264	7939·272	**
2	Difference between 'means regression' and 'average within ages regressions'	0·000073	1	0·000073	—	—
3	Deviations of means about 'means regression'	0·005897	2	0·002948	1·635	N.S.
4	Between adjusted subgroup means	0·005970	3	0·001990	1·122	N.S.
5	Between subgroup regression coefficients	0·010366	3	0·003455	1·948	N.S.
6	Total deviations about subgroup regressions	0·296204	167	0·001774	.	.
7	Average within subgroups regression	0·306570	170	0·001803	.	.
8	Deviations about total regression	0·312540	173	0·001807	.	.
9	Total	14·658804	174	.	.	.

Line 1 is tested against line 8.

Lines 2 and 3 are tested against line 7.

Lines 4 and 5 are tested against line 6.

** indicates significance at 1 % probability level.

* indicates significance at 5 % probability level.

N.S. indicates not significant.

— indicates mean square less than that against which it is tested.

TABLE 15. SUMMARY OF THE ANALYSES OF VARIANCE TESTING THE DIFFERENCES IN LENGTH–WEIGHT RELATION BETWEEN AGE-GROUPS OF LONG ROUGH DABS IN 1954

	Females						Males	
	Immature		Maturing		Spent			
Source	2. iii.	2. vi.	2. iii.	25. xi.	8. iv.	2. vi.	2. vi.	9. ix.
Due to total regression	**	**	**	**	**	**	**	**
Difference between 'means regression' and 'average within subgroups regression'	—	—	—	N.S.	—	—	—	—
Deviations of means about 'means regression'	N.S.	**	N.S.	N.S.	—	—	—	—
Between adjusted subgroup means	N.S.	*	—	N.S.	—	—	—	—
Between subgroup regression coefficients	N.S.	—	—	—	—	—	N.S.	—

TABLE 16. SUMMARY OF THE ANALYSES OF VARIANCE TESTING THE DIFFERENCES IN LENGTH–WEIGHT RELATION OF GROUPS OF LONG ROUGH DABS IN SUBGROUPS BY DATES OF CAPTURE

	Females			
Source	Immature	Maturing	Spent	Males
Due to total regression	**	**	**	**
Difference between 'means regression' and 'average within subgroups regression'	**	**	**	N.S.
Deviations of means about 'means regression'	**	**	**	**
Between adjusted subgroup means	**	**	**	**
Between subgroup regression coefficients	**	N.S.	—	N.S.

TABLE 17. REGRESSION COEFFICIENTS (*b*) OF THE REGRESSIONS OF LOG WEIGHT ON LOG LENGTH TOGETHER WITH THEIR 95% FIDUCIAL LIMITS

	Females									Males		
	Immature			Maturing			Spent					
Date	Lower limit	*b*	Upper limit	Lower limit	*b*	Upper limit	Lower limit	*b*	Upper limit	Lower limit	*b*	Upper limit
1953 Oct.	3·18	3·36	3·54	2·25	2·75	3·24	—	—	—	2·75	3·16	3·57
Nov.	3·02	3·16	3·30	3·02	3·25	3·48	—	—	—	3·10	3·38	3·66
Dec.	3·01	3·17	3·32	2·91	3·19	3·46	—	—	—	2·23	3·18	4·12
1954 Jan.	3·20	3·30	3·40	3·00	3·20	3·41	—	—	—	2·92	3·55	4·18
Feb.	3·20	3·39	3·57	2·86	3·20	3·54	—	—	—	—	—	—
Mar.	3·32	3·40	3·47	2·98	3·15	3·32	—	—	—	1·96	3·01	4·06
Mar.	3·18	3·37	3·55	1·68	2·36	3·04	2·65	3·08	3·52	—	—	—
Apr.	3·08	3·28	3·47	—	—	—	3·02	3·31	3·59	—	—	—
May	3·21	3·28	3·35	—	—	—	3·17	3·34	3·51	2·39	3·97	5·56
June	3·18	3·25	3·32	—	—	—	2·97	3·11	3·25	2·75	3·00	3·25
July	2·82	2·98	3·14	—	—	—	2·70	3·33	3·95	2·82	3·11	3·40
Aug.	2·83	2·96	3·09	2·93	3·09	3·26	2·48	3·04	3·61	2·71	2·92	3·14
Sept.	3·01	3·16	3·31	2·71	3·19	3·67	—	—	—	2·65	2·99	3·32
Oct.	2·78	2·99	3·19	2·86	3·15	3·43	—	—	—	2·71	3·35	3·99
Nov.	2·87	2·99	3·10	2·87	2·98	3·10	—	—	—	2·41	2·84	3·26
1955 Jan.	3·04	3·11	3·18	2·86	3·12	3·38	—	—	—	2·25	2·89	3·54
Feb.	3·21	3·32	3·44	2·86	3·03	3·21	—	—	—			
Mar.	3·13	3·23	3·33	2·49	3·07	3·66	—	—	—			
Apr.	3·09	3·14	3·19	—	—	—	2·85	3·13	3·42	—	—	—
Total	3·14	3·17	3·20	3·04	3·11	3·18	3·04	3·14	3·23	2·95	3·07	3·18

TABLE 18. SUMMARY OF ANALYSIS OF VARIANCE TESTING DIFFERENCES OF REGRESSIONS OF LOG WEIGHT ON LOG LENGTH OF THE MAIN GROUPS (SEX AND MATURITY STAGE) OF LONG ROUGH DABS

Source	Significance
Due to total regression	**
Difference between ‘means regression’ and ‘average within groups regression’	*
Deviations of means about ‘means regression’	**
Between adjusted group means	**
Between group regression coefficients	N.S.

TABLE 19. THE EXPECTED WEIGHTS (g) FOR THE DIFFERENT MATURITY STAGES OF LONG ROUGH DABS 20·0 cm IN LENGTH FOR EACH MONTH OF CAPTURE, WITH THEIR 95% FIDUCIAL LIMITS

Females

	Immature			Maturing			Spent		
Date	Lower limit	$\hat{W}$	Upper limit	Lower limit	$\hat{W}$	Upper limit	Lower limit	$\hat{W}$	Upper limit
1953 Oct.	53·70	55·34	57·02	59·84	61·94	64·12	—	—	—
Nov.	55·08	57·02	59·02	60·81	62·52	64·27	—	—	—
Dec.	54·08	55·85	57·68	58·75	60·81	62·95	—	—	—
1954 Jan.	53·33	54·70	56·10	58·34	59·98	61·66	—	—	—
Feb.	51·76	53·95	56·23	56·89	59·70	62·66	—	—	—
Mar.	54·20	54·95	55·72	58·21	59·57	60·95	—	—	—
Mar.	48·53	50·23	52·00	43·85	49·77	56·49	40·09	43·25	46·67
Apr.	48·53	50·35	52·24	—	—	—	42·36	44·36	46·45
May	47·21	47·95	48·75	—	—	—	41·98	43·35	44·77
June	48·98	49·77	50·58	—	—	—	45·92	47·10	48·31
July	51·17	52·97	54·83	—	—	—	44·77	48·64	52·84
Aug.	48·75	50·23	51·76	51·76	52·97	54·20	45·50	48·08	50·82
Sept.	51·76	53·21	54·70	51·64	54·45	57·41	—	—	—
Oct.	49·09	51·29	53·58	54·33	56·10	57·94	—	—	—
Nov.	52·36	53·70	55·08	56·36	57·41	58·48	—	—	—
1955 Jan.	51·29	52·12	52·97	53·21	55·59	58·08	—	—	—
Feb.	50·47	51·64	52·84	60·53	61·80	63·10	—	—	—
Mar.	50·00	51·05	52·12	52·36	55·98	59·84	—	—	—
Apr.	49·77	50·35	50·93	—	—	—	44·16	48·19	52·60

	Males			Maturing females without gonads		
Date	Lower limit	$\hat{W}$	Upper limit	Lower limit	$\hat{W}$	Upper limit
1953 Oct.	43·05	56·75	74·82	55·59	60·39	65·61
Nov.	54·45	61·24	68·87	58·61	60·53	62·52
Dec.	35·89	56·75	89·74	56·23	58·48	60·81
1954 Jan.	45·50	59·84	78·70	53·46	55·21	57·02
Feb.	—	—	—	50·47	53·95	57·68
Mar.	36·73	52·97	76·38	50·47	51·40	52·36
Mar.	—	—	—	34·12	39·54	45·81
Apr.	—	—	—	—	—	—
May	33·73	51·76	79·43	—	—	—
June	41·11	45·19	49·66	—	—	—
July	47·42	52·60	58·34	—	—	—
Aug.	45·19	48·42	51·88	50·93	52·24	53·58
Sept.	47·42	53·21	59·70	49·55	53·46	57·68
Oct.	43·15	56·36	73·62	48·19	54·58	61·80
Nov.	43·25	51·88	62·23	49·66	52·97	56·49
1955 Jan.				49·43	52·00	54·70

TABLE 20. DISTRIBUTION OF PERCENTAGE MATURE FEMALE FISH IN GROUPS CLASSIFIED BY AGE AND LENGTH. ONLY PERCENTAGES BASED ON MORE THAN TEN FISH GIVEN

Age-group ...	1+		2+		3+		4+		5+	
Year	53–54	54–55	53–54	54–55	53–54	54–55	53–54	54–55	53–54	54–55
Length-group										
11	—	0	16·74	—	—	—	—	—	—	—
14	—	—	13·05	0	—	—	—	—	—	—
17	—	—	15·34	20·27	39·58	—	—	—	—	—
20	—	—	—	30·46	50·77	54·76	58·89	70·54	—	—
23	—	—	—	—	46·32	44·14	57·48	72·74	90·00	—
26	—	—	—	—	—	—	62·58	55·49	—	90·00

TABLE 21. DISTRIBUTION OF PERCENTAGE MATURE FEMALE FISH IN GROUPS CLASSIFIED BY AGE AND WEIGHT. ONLY PERCENTAGES BASED ON MORE THAN TEN FISH GIVEN

Age-group ...	1+		2+		3+		4+		5+	
Year	53–54	54–55	53–54	54–55	53–54	54–55	53–54	54–55	53–54	54–55
Weight-group										
10	0	—	0	13·94	—	—	—	—	—	—
40	—	—	20·09	15·79	55·67	39·23	—	—	—	—
70	—	—	40·22	—	51·18	47·41	72·44	42·13	—	—
100	—	—	—	—	44·20	54·76	70·91	61·14	—	73·26
130	—	—	—	—	—	—	56·54	67·78	—	—
160	—	—	—	—	—	—	—	71·56	—	—

TABLE 22. ANALYSIS OF VARIANCE OF PERCENTAGE MATURITY WITH LENGTH, WEIGHT AND AGE. ONLY PERCENTAGES BASED ON MORE THAN TEN INDIVIDUALS ANALYSED

Source	Sums of squares	Degrees of freedom	Mean square	Variance ratio	Significance
Between ages within lengths	3244·2618	7	463·466	F=10·2	Significant at 1 %
Between lengths within ages	570·1470	8	71·268	F=1·6	Not significant
Within ages and lengths	316·5692	7	45·224	—	—
Between ages within weights	1537·4635	6	256·244	F=2·3	Not significant
Between weights within ages	900·0838	7	128·583	F=1·2	Not significant
Within ages and weights	874·6500	8	109·331	—	—

TABLE 23. ANALYSIS OF REGRESSION OF GONAD WEIGHT ON LENGTH, AND OF GONAD WEIGHT ON WEIGHT FOR DIFFERENT AGES, FOR DATA OF TWO DATES

	Length (cm)		Weight (g)	
Source	15. ii.	2. iii.	15. ii.	2. iii.
Due to total regression	**	**	**	**
Difference between 'means regression' and 'average within age regression'	N.S.	N.S.	N.S.	*
Deviations of means about 'means regression'	N.S.	—	—	—
Between adjusted means	N.S.	—	N.S.	N.S.
Between regression coefficients	N.S.	**	N.S.	N.S.

TABLE 24. THE VALUES OF THE COEFFICIENTS FOR THE REGRESSIONS OF LOG GONAD WEIGHT ON LOG LENGTH AND ON LOG WEIGHT

	Length (cm)		Weight (g)	
Age	15. ii.	2. iii.	15. ii.	2. iii.
2+	—	3·408	—	0·881
3+	2·747	3·175	0·862	0·880
4+	5·845	2·339	2·082	0·650
5+	4·876	−0·797	1·092	0·076
Total regression	3·829	3·162	1·242	0·954

TABLE 25. THE EXPECTED GONAD WEIGHT OF A FEMALE LONG ROUGH DAB OF 69·98 g (THE GRAND MEAN WEIGHT)

	1953–54 (g)	1954–55 (g)
Aug.	—	0·64
Sept.	—	0·81
Oct.	1·88	1·61
Nov.	2·36	2·75
Dec.	2·99	—
Jan.	5·08	4·66
Feb.	6·70	8·13
Mar.	9·75	12·39
Mar.	10·50	—

TABLE 26. SUMMARY OF ANALYSES OF REGRESSIONS OF FECUNDITY ON LENGTH AND ON WEIGHT OF FISH IN DIFFERENT AGE-GROUPS

	Length		Weight	
Source	15. ii.	2. iii.	15. ii.	2. iii.
Due to total regression	**	**	**	**
Difference between 'means regression' and 'average within ages regressions'	—	—	—	N.S.
Deviations of means about 'means regression'	—	—	—	—
Between adjusted age means	—	—	—	N.S.
Between subgroup regression coefficients	N.S.	N.S.	N.S.	N.S.

TABLE 27. THE ABOUT REGRESSION MEAN SQUARES FROM THE ANALYSES OF THE REGRESSIONS OF LOG FECUNDITY (*F*) ON THE LOGS OF AGE (*A*) LENGTH (*L*) WEIGHT (*W*) AND GONAD WEIGHT (*G*)

F on	15. ii. 54	2. iii. 54
A	0·0949	0·1057
L	0·0233	0·0534
W	0·0161	0·0543
G	0·0272	0·0541
A and *L*	0·0240	0·0540
A and *W*	0·0168	0·0542
A and *G*	0·0195	0·0539
L and *W*	0·0166	0·0533
L and *G*	0·0128	0·0443
W and *G*	0·0103	0·0433
L, *W* and *G*	0·0108	0·0438

TABLE 28. EQUATIONS FOR ESTIMATING THE FECUNDITY (*F*) FROM LENGTH (*L*) WEIGHT (*W*) AND GONAD WEIGHT (*G*) OF LONG ROUGH DABS

Factor	Equation	Standard error of estimate (eggs)
	15. ii. 54	
L	$\hat{F}=1{\cdot}4333\ L^{3{\cdot}5533}$	±39,070
W	$\hat{F}=626{\cdot}71\ W^{1{\cdot}1388}$	±38,880
W and *G*	$\hat{F}=1674\ W^{0{\cdot}7699}\ G^{0{\cdot}2970}$	±38,750
L, *W* and *G*	$\hat{F}=3709\ L^{0{\cdot}2077}\ W^{0{\cdot}8350}\ G^{0{\cdot}2949}$	±38,750
	2. iii. 54	
L	$\hat{F}=7{\cdot}154\ L^{3{\cdot}0621}$	±26,890
W	$\hat{F}=1603\ W^{0{\cdot}9461}$	±26,910
W and *G*	$\hat{F}=3458\ W^{0{\cdot}5192}\ G^{0{\cdot}4481}$	±26,700
L, *W* and *G*	$\hat{F}=2111\ L^{0{\cdot}2556}\ W^{0{\cdot}4486}\ G^{0{\cdot}4403}$	±26,720

TABLE 29. THE 95 % FIDUCIAL LIMITS OF THE REGRESSION COEFFICIENTS (*b*) OF THE REGRESSIONS OF LOGARITHMS OF FECUNDITY ON THE LOGARITHMS OF AGE, LENGTH, WEIGHT AND GONAD WEIGHT

	15. ii. 54			2. iii. 54			
Factor	*b*	Lower limit	Upper limit	*b*	Lower limit	Upper limit	Remarks
Age	1·0033	0·6212	1·3854	1·0328	0·8894	1·1762	Linear
Length (mm)	3·5533	3·4304	3·6762	3·0621	2·8337	3·2905	>Cubic
Weight (g)	1·1388	1·1058	1·1718	0·9461	0·9235	0·9686	*ca.* linear
Gonad weight (g)	0·6907	0·6645	0·7169	0·8117	0·7806	0·8428	<Linear

TABLE 30. THE LENGTH, WEIGHT, GONAD WEIGHT, AGE AND EGG COUNTS OF FEMALE LONG ROUGH DABS

Fish no.	Total length (cm)	Flesh wt. (g)	Ovary wt. (g)	Age	Egg count				Fecundity estimate
					1	2	3	4	
15 February 1954									
324	295	175·0	26·5	4+	940	1069	994	1183	209,300
325	230	84·0	10·5	3+	509	510	518	525	103,100
326	230	81·0	8·5	3+	471	430	510	408	90,950
327	245	106·5	11·5	3+	505	584	561	558	110,400
329	230	90·0	10·5	3+	515	519	520	506	103,000
330	250	101·5	12·5	3+	563	606	667	707	127,150
333	245	96·5	13·5	4+	703	730	814	731	148,900
334	240	95·0	11·5	4+	717	682	503	520	121,100
335	260	117·0	12·5	4+	767	700	693	772	146,600
337	210	65·0	4·5	3+	345	295	359	346	67,250
338	235	85·5	12·5	3+	437	406	412	508	88,150
339	255	106·0	7·5	4+	667	610	627	600	125,200
341	230	84·5	9·0	3+	538	491	513	450	99,600
342	250	118·5	10·5	3+	570	588	654	641	122,650
343	240	89·0	10·0	3+	392	383	401	553	86,450
348	215	69·5	6·0	4+	448	421	380	388	81,850
351	225	68·0	2·5	4+	268	267	342	273	57,500
354	240	95·0	9·5	4+	492	550	449	475	98,300
355	245	96·5	11·0	3+	653	573	587	646	122,950
359	290	172·0	24·0	5+	1340	1300	1245	1078	248,150
360	265	123·0	17·5	4+	967	1076	869	912	191,200
363	305	226·5	21·5	5+	1383	1256	1240	1281	258,000
365	240	78·0	7·5	5+	436	348	388	318	74,500
366	220	66·5	7·5	3+	356	310	396	307	68,450
370	200	57·0	9·0	3+	415	346	411	448	81,000
Mean	243·6	101·88	11·5	3·6		606·34			121,268
2 March 1954									
379	190	35·0	8·5	3+	253	255	199	229	46,800
380	220	69·0	5·0	3+	354	331	366	348	69,950
381	235	83·5	13·0	4+	459	625	643	573	115,000
383	210	53·5	12·5	3+	489	399	537	347	88,600
385	245	85·5	13·5	4+	636	646	604	478	118,200
386	215	67·0	12·0	3+	558	504	447	461	98,500
387	195	50·5	6·5	3+	208	172	186	239	40,250
388	205	101·5	12·0	4+	374	464	474	458	88,500
389	220	69·0	13·5	4+	506	535	424	510	98,750
390	250	101·5	18·0	4+	873	773	780	681	155,350
391	200	47·5	5·5	3+	244	276	245	221	49,300
392	175	28·5	5·5	2+	247	242	181	202	43,600
398	290	156·0	10·5	5+	950	1025	1057	992	201,200
399	205	52·5	9·5	3+	349	345	382	286	68,100
400	215	65·5	14·0	4+	436	444	373	476	86,450
401	195	47·5	6·5	3+	250	233	216	191	44,500
402	225	65·5	11·0	4+	395	310	387	413	75,250
403	180	39·0	3·5	2+	208	219	252	213	44,600
404	200	56·0	11·0	4+	302	396	386	348	71,600
420	190	44·5	8·5	2+	266	214	285	237	50,100
421	225	73·0	13·0	3+	537	582	593	523	111,750
422	225	72·0	10·0	3+	474	537	543	506	103,000
423	190	43·5	3·0	3+	168	181	201	151	35,050
424	190	39·0	7·0	3+	189	238	172	227	41,300
425	225	77·5	11·0	3+	635	498	546	517	109,800
426	210	58·0	10·5	3+	363	435	452	360	80,500
427	210	60·5	11·5	3+	540	531	467	550	104,400
428	205	55·5	8·5	4+	416	335	363	345	72,950
429	230	73·5	11·5	3+	489	458	506	462	95,750
430	185	35·5	3·5	2+	218	190	187	177	38,600
431	200	53·5	6·5	2+	348	322	306	324	65,000
432	200	45·5	8·5	4+	401	402	428	325	77,800
433	210	64·0	11·5	3+	551	509	594	582	111,800
469	200	54·5	7·0	2+	299	370	321	312	65,100

TABLE 30 (*continued*)

Fish no.	Total length (cm)	Flesh wt. (g)	Ovary wt. (g)	Age	Egg count				Fecundity estimate
					1	2	3	4	
470	185	40·0	3·5	2+	161	188	174	173	34,800
471	210	55·5	10·0	3+	348	298	379	367	69,600
472	240	83·0	10·0	3+	485	520	509	546	103,000
473	200	44·0	10·5	3+	375	420	415	381	79,550
474	230	68·5	13·5	3+	517	514	503	453	99,350
475	205	52·0	7·0	4+	267	253	284	271	53,750
476	185	42·0	6·5	3+	1107	1112	1050	1057	216,300
477	175	34·0	4·5	3+	180	187	197	174	36,900
482	170	29·0	3·5	2+	166	197	210	199	38,600
483	180	32·5	6·5	2+	223	259	291	261	51,700
485	210	63·5	7·5	2+	288	336	363	347	66,700
486	215	59·0	9·5	4+	465	430	449	368	85,600
487	220	74·0	8·5	4+	368	396	369	408	77,050
488	235	90·0	14·5	3+	534	478	512	430	97,700
489	270	155·0	15·5	4+	258	270	249	273	52,500
490	200	60·0	7·5	3+	284	313	381	402	69,000
491	210	50·0	8·5	3+	280	292	351	292	60,750
492	195	42·5	7·0	3+	290	287	269	320	58,300
493	210	51·5	8·0	4+	296	266	302	305	58,450
494	180	39·0	6·0	2+	308	286	350	327	63,550
495	185	38·5	5·5	3+	271	270	346	280	58,350
496	185	40·0	6·0	3+	248	236	198	189	43,550
497	165	25·5	3·5	2+	170	191	142	141	32,200
503	245	102·0	18·0	3+	700	801	940	794	161,750
504	260	119·0	15·5	4+	1027	908	922	957	190,700
505	245	96·0	12·0	3+	700	701	701	698	140,000
506	240	93·0	12·5	4+	589	598	543	629	117,950
507	230	79·0	10·0	3+	549	510	613	492	108,200
512	220	73·5	10·5	3+	449	483	442	385	87,950
513	220	68·0	10·5	2+	377	426	380	454	81,850
514	220	68·5	11·0	3+	426	475	434	439	88,700
515	205	56·0	10·5	2+	484	421	411	454	88,500
516	215	58·0	9·0	3+	344	366	329	352	69,550
517	180	34·5	6·0	2+	236	170	229	218	42,650
518	185	41·5	5·5	2+	242	200	262	226	46,500
519	170	30·0	5·0	2+	154	172	151	175	32,600
520	170	33·0	5·0	2+	219	180	146	178	36,150
521	170	29·0	3·5	2+	150	148	130	169	29,850
522	165	27·0	3·0	2+	133	117	145	168	28,150
552	290	173·0	27·0	5+	1050	972	1055	953	201,500
553	270	107·5	17·0	5+	770	674	814	674	146,600
554	245	96·0	21·0	5+	729	662	792	610	139,650
555	255	97·5	15·0	5+	552	552	518	558	109,000
556	215	74·5	6·5	3+	347	374	360	391	73,600
557	210	62·0	6·0	3+	365	372	364	329	71,500
558	200	44·5	8·0	4+	365	316	301	326	65,400
559	195	50·5	7·0	2+	401	378	265	349	69,650
560	205	60·0	10·5	3+	476	442	387	469	88,700
561	210	55·5	7·5	3+	311	271	314	263	57,950
562	230	73·0	14·0	3+	415	403	414	445	83,850
563	225	78·0	8·0	3+	508	457	355	429	87,450
564	265	121·5	22·0	5+	872	786	752	850	163,000
565	230	70·5	7·0	3+	454	371	385	408	80,900
566	215	57·5	9·0	4+	280	288	302	268	56,900
567	240	90·5	11·5	4+	553	584	588	538	113,150
568	190	40·0	4·0	3+	272	241	199	260	48,600
569	200	59·0	4·5	2+	307	307	346	334	64,700
Mean	21·16	63·87	9·44	3·1	409·22				81,844
15 March 1954									
669	155	21	4·0	·	102	158	136	121	25,850
30 March 1954									
691	165	28	5·5	2+	197	202	156	191	37,300
692	150	18	4·0	2+	127	139	118	130	25,700

APPENDIX 1

THE ANALYSIS OF THE LENGTH–WEIGHT RELATION AND CONDITION

The data used in this analysis were obtained from the samples listed in Table 1. The methods of obtaining the data have been given, but it must be remembered that there is no evidence available as to the selectivity of the trawl with regard to the length–weight relation. We may suppose that the fatter and heavier fish of a given length would be more easily retained by trawl meshes of a given size.

A preliminary scatter diagram (Fig. 1) of length and weight suggested that their relationship was of the form

$$W = aL^n,$$

where W = weight, L = length, a is constant and n an exponent approximately = 3. The analysis of the data could best be accomplished by using logarithms and fitting the straight line regression of log weight on log length,

$$\log W = \log a + n \log W$$

to the data by the method of least squares.

The data were divided into four groups according to sex and maturity as follows: (1) males; (2) immature females (stage 1); (3) maturing females (stage 2); (4) spent females (stage 4). These groups were further divided into subgroups by month of capture. Although it was originally proposed to further divide the subgroups into their respective age-classes, this was not found to be practicable owing to the small numbers that would be in each division. The two largest subgroups from each main group were chosen and a preliminary analysis carried out to see if the length–weight relation differed in the different age-classes. This was carried out by an analysis of covariance in a manner similar to that used by LeCren (1951). For an account of the statistical methods see Snedecor (1946, pp. 318–29) and Mather (1946, pp. 119–28). As an example of the calculations, the data for the immature females in the sample caught on 2 March 1954 are given in Tables 13 and 14. The two most important tests (apart from line 1, Table 14) are those of lines 4 and 5 (Table 14). The first tests the significance of the differences between the expected weights in each subgroup corresponding to the grand mean length. This is therefore a test of the differences in condition between the different subgroups (see next section). The second tests the difference between the subgroup regression coefficients and is therefore a test of the difference in length–weight relation of the subgroups. The other tests are of less interest. Line 3 tests the significance of the regression of the means of the subgroups, while line 2 tests whether there is a significant difference between the average of the subgroup regressions and the regression of their means. It is obvious that if line 3 is significant, line 2 can have no meaning.

Various tests were performed on the data before the regression analyses were carried out, in order to see to what extent the assumptions made were in fact valid. Bartlett's test of homogeneity was applied to the residual variances (Pearson & Hartley, 1954); because in order that the tests of significance may be valid the data must be homogeneous. These tests showed that the data were heterogeneous. The heterogeneity was not removed by considering the age-groups separately and it was thought that Bartlett's test might have revealed non-normality in the log length and log weight frequency distributions. Tests for normality were carried out on a large number of these distributions and the majority were found to be significantly non-normal. It is clear that if the lengths are normally distributed, the weights will not be, if the length–weight relation approximates to $W = aL^3$. Although taking logarithms helps to normalize the weights, it simultaneously tends to skew the lengths. For a critical discussion a considerably more complicated form of analysis is required, and it was not thought that such an analysis was justified with the present data. It should be borne in mind, however, first that the effects of non-normality do not invalidate the estimates of the regression; the equation found is still the 'best straight line' (by the method of least squares) for the data; it is only the tests of significance that are less reliable than would be the case with strictly normal and homogeneous data. Secondly, the effect of non-normality is usually to produce more significant results than would otherwise be found (Cochran, 1947). In this work on Long Rough Dabs it was found (for example in Tables 5 and 6) that the comparisons being tested were mostly either not significant ($P > 0{\cdot}05$) or highly significant ($P < 0{\cdot}01$), and in only a few cases would the conclusions be changed by using the 0·01 level of probability as a basis for rejection of a hypothesis rather than the more usual level of 0·05, and the more severe test may be used for these data.

THE LENGTH–WEIGHT RELATION

The analyses of the regressions of log weight on log length were first carried out on different age-groups of otherwise comparable fish (Table 15). These 'between ages' comparisons were made on the two subgroups from each main group that contained the most individuals. It can be seen that in one case there was no definite trend in the means of the different ages, and in this case too the differences in 'condition' of the fish are possibly significant. It will be noted that in no case was there a significant difference in the length–weight relation of the different ages as shown by the regression coefficients. This evidence suggests that the age-groups may be pooled and the analysis continued to test the differences on different dates of collection.

The results of these analyses are given in Table 16 and the regression coefficients for the regressions are given in Table 17 together with their 95% fiducial limits.

These limits were calculated as

$$b \pm t[1/Sx^2\,(\text{residual s.s.}/n-2)]^{\frac{1}{2}},$$

where t = Student's 't' for $n-2$ degrees of freedom, Sx^2 = sum of squares for length, residual s.s. = residual sum of squares (e.g. from Table 13). In Table 16 it will be noted that only for the immature females are there significant differences between the coefficients for different dates.

It is usual in studies of the length–weight relation of fish to test if the regression coefficients are significantly different from 3, to see if the fish in question obey the 'cube law'. It can be seen from Table 17 that 3 is within the 95% fiducial limits for most subgroups except those of the immature females, for which the coefficients are mostly significantly greater than 3. However, with the total regressions, based on all the points summed over dates, only with the males do the fiducial limits include 3. It is usually assumed that differences in the regression coefficients at different times are not of interest and are only estimates of the overall value. However, the fluctuations in the coefficient may be of interest since they indicate how different size-groups are behaving in their length–weight relation. For example, if all fish are putting on weight and the large fish do so relatively faster, the coefficient increases, whereas if the coefficient decreases it is indicative of relatively faster weight increase by the smaller fish. In this way the study of the regression coefficient should be closely linked with the analysis of the condition.

It has been noted that only the immature females showed significant differences in the regression coefficients of different dates. These are shown graphically in Fig. 2, where it can be seen that there appears to be a yearly cycle in the value of b. The biological significance of this cycle has already been discussed. With maturing and spent Long Rough Dabs the differences in the coefficients are not significant, but even so they may be of interest. The values of the coefficients for the total regression (pooled over dates for each group) are also given in Table 17, and the significance of their differences are shown in Table 18. The data for the immature fish are included even though not homogeneous.

In Table 16 can be found the significance of the differences in the adjusted means for the different subgroups (dates) of the main groups of Long Rough Dabs. These adjusted means for each subgroup are the expected mean weights of fish of the grand mean length (for each main group). They are, therefore, a test of the condition between the subgroups of fish and it will be noticed that in each case the differences are significant. Within each group the adjusted weights are comparable, but in order to compare the condition of the different groups one with another it is necessary to adjust the weights not to the mean length of the group but to a length more suitable for all the groups.

An arbitrary length of 20 cm was chosen as being conveniently within the range of all the groups and sufficiently near the grand mean length. While male Long Rough Dabs as large as 20 cm are rare, this length is at the lower end of the range of mature females. The weights expected ($\hat{W}$) for fish of 20 cm calculated from the regression equations for each month are given in Table 19 together with their 95 % fiducial limits, and are shown diagrammatically in Fig. 3. The differences found to be significant in Table 6 can be taken as equally applying to these variations. A discussion of the results is given earlier in the paper.

The expected weights are given by

$$\hat{W} = \overline{W} . L^b / \bar{l}^b,$$

where $L = 20$ cm, and $\overline{W}$ and $\bar{l}$ are the arithmetic means of the weights and lengths obtained from the raw data. This equation is derived from

$$\log \hat{W} = \log \overline{W} + b(\log L - \log \bar{l}),$$

but for estimating the weight from the lengths, the antilog of log $\hat{w}$ gives a biased result since it is based on the mean of the logarithms (= geometric mean) and the arithmetic mean should be used (M. Bagenal, 1955). The 95 % fiducial limits of estimated weight ($\hat{W}$) are given by

$$\text{Antilog } [\log \hat{W} \pm t[(1/n + (L - \bar{l})^2/Sx^2) \text{ residual s.s.}/n-2]^{\frac{1}{2}}].$$

In this case the limits may be calculated on the logarithmic values and the antilogarithm taken to give the limits in grams.

APPENDIX 2

THE ANALYSIS OF MATURITY AND AGE, LENGTH AND WEIGHT

The data for the percentage of mature fish were grouped into two tables for length and weight (Tables 20 and 21). It will be seen that in some cases the two years 1953–54 and 1954–55 provided pairs of percentages of the same age and length. The variability between these pairs provided a standard against which it was possible to test (after a transformation of percentages into angles) (Snedecor, 1946, p. 447 and table 16·8) the variability between ages within length-groups, and between length-groups within ages; and similarly for ages and weights.

The result of this test is given in Table 22, where it is seen that the variability between different ages with the same length is significantly greater than that between years, though the variability between length-groups of the same age is not.

A comparable analysis for age and weight is also given in Table 22. Here the variance between ages, although double that of the 'between weights' is not statistically significantly greater than that of the 'between pairs' variance.

APPENDIX 3

THE ANALYSES OF GONAD WEIGHT

The effect of the age of the female Long Rough Dab on gonad development was examined in preliminary regression analyses of the logarithms of the gonad weights on lengths and on weights for different ages carried out on the data of 15 February and 2 March 1954. The results of these analyses are summarized in Table 23. Full details of method and data are given in Appendix 1.

The coefficients for the regressions of gonad weight on length for the different ages were found to differ significantly for the 2 March data. To a large extent this variability was due to the inclusion of the data from the six 5-year-old fish, which were widely scattered; but even without these fish the coefficients were significantly different at the 5 % level. Since in the analysis of regression on weight the regression coefficients were not significantly different it is probably safe to ignore this anomalous result. The values for the regression coefficients are given in Table 24. There is no clear trend in the coefficients with increasing age and those for the regressions of gonad weight on weight may be taken to indicate a linear relationship; that is, the data suggest that there is no difference in the gonad weight/flesh weight ratio in the different age-groups.

The analyses summarized in Table 23 indicate that either length or weight may be used in the further analysis of the gonad weight annual cycle, and that the different ages may be pooled. The standard error of estimates of gonad weight are very comparable for the length and weight regressions, being 0·112 g for the former and 0·119 g for the latter for the March data, and 0·146 g for both length and weight for February. For further analysis the regression of log gonad weight on log weight was used. The values for the regression coefficients did not appear to change from month to month in any ordered manner, showing there was no obvious difference in the time of gonad development of fish of different sizes. The adjusted gonad weights were calculated for fish of a grand mean weight of 69·98 g and are shown in Table 25 and Fig. 6.

APPENDIX 4

THE ANALYSIS OF FECUNDITY

In Fig. 9 the fecundity and length data are given as a scatter diagram for the 119 observations, and in Fig. 10 the fecundity and weight are shown. While the latter figure suggests that the relation of fecundity and weight may be linear, Fig. 9 suggests that the relation with length would be of the form

$$F = aL^n,$$

or

$$\log F = \log a + n \log L,$$

where F = fecundity, L = length and a and n are a constant and exponent to be obtained from the data. Both these suggestions were examined by analyses of the regressions of logarithm of fecundity on the logarithm of the independent factor. First, however, analyses of covariance were carried out to determine whether the relations of fecundity and length and weight changed significantly with age. These analyses were essentially similar to those shown in Tables 3 and 4 for the length–weight relation. The results of these analyses are shown in Table 26.

The regressions analysed were those of log fecundity on log length and log weight; the values for the fecundity were the arithmetic means of the four original estimates for each fish. Since the differences between the ages were not significant, the data were pooled and the regressions of log fecundity on log age, length, flesh weight and gonad weight were analysed. Later multiple regression analyses of fecundity on combinations of these characters were also carried out. In each analysis the 'due to regression' mean square was tested against the 'about regression' variance to test the validity of the regression in question. From the four estimates of the fecundity for each fish, the 'within fish' variance could be calculated ($= \Sigma F_i^2 - 4\Sigma \bar{F}^2$, where Fi for $i = 1, 2, 3$ and 4 are the logarithms of the four fecundity estimates and $\bar{F}$ their mean) and was taken as a measure of the errors inherent in the counting and subsampling method. This 'within fish' variance is the appropriate mean square for testing the 'about regression' variance, since it is hoped to reduce the variability about the regression plane (by the most suitable combination of characters for estimation) until it is no longer significantly larger than the variability of the laboratory technique. The 'within fish' variance, however, was not large (0·0019 with 273 degrees of freedom, on 2 March 1954 and with 75 degrees of freedom on 15 February 1954), and the 'about regression' variances were always significantly greater than this. The more highly statistical method outlined above for finding the most suitable factors for use in estimating fecundity seems more satisfactory than that used by Simpson (1951) for Plaice. Although Simpson also counted four subsamples of the eggs from each fish, he used these not as a measure of his experimental error, but only to arrive at a more reliable estimate of the fecundity (the standard deviation of the estimate would be reduced by $\sqrt{4}$ of that of a single count). His estimate of the errors in the laboratory method was based on his count of forty subsamples taken specially for the purpose. With the methods given here one may easily measure which factors are the most reliable for estimating fecundity, and test their significances.

After the analyses of covariance and tests of significance had been carried out, the regression equations for estimation were transformed back from logarithms to actual values, and the standard errors of estimate of the real fecundity were calculated. This (by rearrangement of equation 5, M. Bagenal, 1955) is given by the standard error of estimate

$$= \sigma_{\hat{F}} = 200\bar{F}[\text{antilog } (\sigma_y^2/\log_{10} e) - 1]^{\frac{1}{2}},$$

where $\sigma_y^2 =$ the total 'about regression' residual variance in logs and $\bar{F} =$ the arithmetic mean fecundity from all the estimates. Using the above formula to obtain the 'between counts' errors for the two sets of data one obtains $\sigma_{\text{counts}} = 61{\cdot}240$ for the mean count of 606·34 eggs for 15 February and $\sigma_{\text{counts}} = 41{\cdot}331$ for the mean count of 409·22 eggs of the 2 March collection. These both correspond to a coefficient of variation of 0·1010 or 10·10% (since the within fish variance was the same for both dates); this closely approximates to the value obtained from the 40 replicate counts. Of the standard errors of estimate given later for each regression equation ± 12,248 eggs and ± 8266 eggs can be accounted for by the variability in laboratory method of the February and March collections respectively.

The 'about regression' mean squares, obtained from the analyses of the regressions and multiple regressions of log fecundity on the logs of age, length, weight and gonad weight are given in Table 27. In all cases these are significantly greater than the 'within fish' mean square, showing that the great variability between fish cannot be due to experimental technique alone.

Of the single factors, age alone is of little use for estimating fecundity and, indeed, for the 15 February data, the 'due to regression' mean square is only significant at the 5% level. Furthermore, the addition of age to the other factors does not produce any significant improvement, except to gonad weight with the February data. Age is not therefore considered in any three factor analyses.

On neither date does the addition of length to the regression of fecundity on weight significantly decrease the 'about regression' sums of squares, and for the March data weight does not significantly improve length alone for prediction purposes. In all other cases the addition of a second variate significantly reduces the 'about regression' sums of squares.

From the two variate analyses it is seen that for both dates weight and gonad weight together are the best for fecundity prediction purposes, and the addition of length as a third variate does not produce a significant improvement. The most useful equations for predicting fecundity are given in Table 28, together with the standard errors of estimate. The equations were obtained, in the case of the relation with length for example, in the form

$$\hat{F} = 200\bar{f} \,.\, L^b / \bar{l}^b,$$

where $\hat{F}$ is the expected fecundity for a given length (L) and $\bar{f}$ and $\bar{l}$ are the arithmetic means of the egg counts and lengths obtained from the raw data. This is similar to the equation used for estimating 'condition' from the length–weight relation (p. 372).

The choice of the measurement to be used for estimating fecundity should not of course be dictated solely by a consideration of Table 27. The lengths of fish are so much easier and quicker to obtain than the corresponding weights and gonad weights that if time is limited and the fish are not, greater accuracy in estimating the mean fecundity might be obtained by measuring length alone since the standard error of estimate would be reduced by increasing the sample size.

The values of the fiducial limits at the 95 % probability level for the coefficients of the regressions of the logarithms are given in Table 29.

A further aspect of the relation of fecundity to length and weight was examined by a consideration of the correlation of fecundity and condition. The correlation ($r = 0{\cdot}12$) between the log condition ($= \log W - \log \hat{W}$) and the logarithms of the deviations of the fecundities from their expected values ($= \log F - \log \hat{F}$) was not found to be significant ($\hat{W}$ = expected weight and $\hat{F}$ = expected fecundity). In other words, it was found that there was no association between the deviations of the fecundities from the line of their regression on length, and the deviations of the weights from their regression line on length.

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ANNUAL VARIATIONS IN FISH FECUNDITY

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The variation in the fecundity of a fish species from year to year has not received much attention, although the changes in the numbers of young fish in the plankton are well documented for the Plymouth area (Russell 1930–47; Corbin, 1948–51). The most important work considering fecundity fluctuations is that of Simpson (1951) who, working on Plaice, *Pleuronectes platessa* L., concluded that during 1947, 1948 and 1949 there was a steady decrease in the number of eggs laid, this being due to a decrease in the mean size of the spawning fish which he showed to be correlated with the egg numbers. Simpson's interest in the fecundity was to provide a more accurate estimate for determining the number of plaice in the total population from planktonic egg surveys similar to the work of Buchanan-Wollaston (1923). He did not consider in detail whether the fecundity for a given length might change from year to year. It is this problem which will be considered here. Estimates of fecundity of Long Rough Dabs, *Hippoglossoides platessoides* (Fabr.), will be analysed, and Simpson's Plaice data will also be considered.

MATERIAL AND METHODS

The fecundity of the fish is defined, for the purposes of this paper, as the number of eggs in the ovary before spawning.

The data for Plaice are taken from the admirably complete appendix I given by Simpson (1951), where full details are presented for Plaice caught in the North Sea Southern Bight during 1947/48 and 1948/49.

The data for Long Rough Dabs for 1954 are given in table 30, appendix 4, of Bagenal (1957) and for 1955 and 1956 in the Appendix of this paper (p. 382). The details of sampling methods and subsequent laboratory treatment of the fish, together with particulars of the storage, subsampling, counting of the eggs and statistical analysis are all given in the earlier paper (Bagenal, 1957).

I would like to thank Miss Sheila Morris who counted the eggs and did much of the computation, my wife for statistical advice and the master and crew of M.V. *Calanus* who caught the fish.

LONG ROUGH DAB FECUNDITY IN 1954, 1955 AND 1956

The mean length, weight, age and fecundity are given in Table 1 for fish caught in 1954, 1955 and 1956, together with the expected weight ($\hat{W}$) of a fish 22 cm long. $\hat{W}$ has been calculated from the log length–log weight relation and may be taken as a measure of the condition of the fish (Le Cren, 1951; Bagenal, 1957).

The expected fecundity ($\hat{F}$) of a 22 cm Long Rough Dab is also given for each year and has been calculated from the log length–log fecundity relation (Bagenal, 1957). The results of the statistical analysis are given in Table 2 and show that the fecundities, even after allowance has been made for the length differences, differ significantly at the 1 % level from year to year. The condition, as shown by expected weights, for fish of 22 cm is also significantly different over the three years, and it is of interest that the ranked order is the same for condition and fecundity.

TABLE 1. SUMMARY OF DATA GIVEN IN TABLE 5

Year	1954	1955	1956
Number of fish	116	12	23
Mean length (cm)	21·85	21·04	22·70
Mean weight (g)	72·06	55·54	79·53
Mean age (years)	3·2	4·2	3·7
Mean fecundity	90,341	68,292	98,339
$\hat{W}$ for 22 cm	73·65	64·32	71·74
$\hat{F}$ for 22 cm	92,238	78,468	89,193

TABLE 2. ANALYSIS OF COVARIANCE OF LENGTH AND FECUNDITY DATA FOR LONG ROUGH DABS IN 1954, 1955 AND 1956

Source	Sum of squares	D.F.	Mean square	F	Significance
Due to total regression	19·043581	1	19·043581	1466·1	**
Difference between 'means regression' and 'average within years regression'	0·034180	1	0·034180	2·67	N.S.
Deviations of means about 'means regression'	0·099176	1	0·099176	7·75	**
Between adjusted fecundity means	0·133356	2	0·066678	5·22	**
Between years regression coefficients	0·043802	2	0·021901	1·71	N.S.
Total deviations about years regressions	7·642091	598	0·012779	—	
Average within years regression	7·685893	600	0·012801	—	
Deviations about total regression	7·819249	602	0·012989	—	
Total	26·862830	603	—		

** indicates significance at 1 % probability level.
* indicates significance at 5 % probability level.
N.S. indicates not significant.
The degrees of freedom are based on four counts for each fecundity estimate.

The large mean square for the deviations of the means about their regression, when contrasted with the very large mean square due to total regression and the smaller mean square after adjustment to a common length, emphasizes the utility of an analysis of covariance based on all the data.

PLAICE FECUNDITY IN 1947/48 AND 1948/49

The fecundity data given by Simpson (1951) for Southern Bight North Sea Plaice caught in 1947/48 and 1948/49 are summarized in Table 3.

The mean weights are based on the gutted weight minus ovary weight, as with the Long Rough Dabs, and the 'condition' ($\hat{W}$) also applies to somatic tissue only. The mean age is calculated assuming the queried ages Simpson gives were correctly assessed; to ignore the doubtful otolith readings would introduce bias since older fish are the most difficult to age.

TABLE 3. SUMMARY OF SIMPSON'S DATA ON PLAICE FECUNDITY IN THE SOUTHERN BIGHT IN 1947/48 AND 1948/49

Year	1947/48	1948/49
Number of fish	169	54
Mean length (cm)	37·14	37·08
Mean weight (g)	515·21	528·37
Mean age (years)	7·28	7·17
Mean fecundity	84,030	87,740
$\hat{W}$ for 37 cm	509·34	525·20
$\hat{F}$ for 37 cm	82,996	87,152

TABLE 4. ANALYSIS OF COVARIANCE OF REGRESSION OF LOG FECUNDITY ON LOG LENGTH

Source	Sum of squares	D.F.	Mean square	F	Significance
Due to total regression	16·348027	1	16·348027	641·80	**
Difference between 'means regression' and 'average within years regression'	0·037850	1	0·037850	1·49	N.S.
Deviations of means about 'means regression'	—	—	—	—	—
Between adjusted fecundity means	0·037850	1	0·037850	1·48	N.S.
Between years regression coefficients	0·007882	1	0·007882	—	—
Total deviations about years regressions	5·583633	219	0·025496	—	—
Average within years regression	5·591515	220	0·025416	—	—
Deviations about total regression	5·629365	221	0·025472	—	—
Total	21·977392	222	—	—	—

The relation of log fecundity to log length has been re-examined by an analysis of covariance, and the results are given in Table 4. A note arising out of this analysis is given in Appendix 2.

The degrees of freedom are based on one fecundity estimate for each fish.

An examination of Tables 3 and 4 show that the decrease in length in the catches over the two years is barely reflected in the data given by Simpson. The general level of fecundity increased, but this is not statistically significant, and Simpson was justified when he pooled the results for the two years. The weights adjusted to a common length are also not significantly different.

DISCUSSION

Simpson was correct in his conclusion that the drop in the mean number of eggs laid per female Plaice in the Southern Bight in 1947, 1948 and 1949 was only due to a decrease in the mean size of the spawning fish. The fecundity adjusted to a given length actually increased, though this was not significant over the two years for which data are given. In Long Rough Dabs from the Clyde area, however, significant changes have been found in the fecundity even after allowance has been made for length differences. If population estimates based on fish egg estimates are made over several seasons one cannot necessarily assume that the fecundity–length relation remains constant. An examination of Tables 1 and 3 shows that fecundity differences cannot be explained by the different age structure of the population.

It may, however, be significant that for the Long Rough Dabs and the Plaice the ranked order of fecundity and condition for the years considered are the same. Within a year (1954) no correlation was found between the condition and fecundity of individual Long Rough Dabs (Bagenal, 1957). Comparisons between years and different localities may help to explain some of the enormous variability in fecundity of otherwise apparently similar fish, and a programme of fecundity estimates of a number of species over several years is being initiated at Millport.

REFERENCES

BAGENAL, M., 1955. A note on the relations of certain parameters following a logarithmic transformation. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 289–96.

BAGENAL, T. B., 1957. The breeding and fecundity of the Long Rough Dab *Hippoglossoides platessoides* (Fabr.) and the associated cycle in condition. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 339–75.

BUCHANAN-WOLLASTON, H. S., 1923. The spawning of Plaice in the southern part of the North Sea in 1913–14. *Fish Invest. Lond.*, Ser. 2, Vol. 5, No. 2, 36 pp.

CORBIN, P. G., 1948–51. The seasonal abundance of young fish. IX–XI. *J. mar. biol. Ass. U.K.*, Vol. 27, pp. 718–22; Vol. 28, pp. 707–12; Vol. 30, pp. 271–6.

LE CREN, E. D., 1951. The length–weight relation and seasonal cycle in gonad weight and condition in the Perch (*Perca fluviatilis*). *J. Anim. Ecol.*, Vol. 20, pp. 201–19.

RUSSELL, F. S., 1930–47. On the seasonal abundance of young fish. I–VIII. *J. mar. biol. Ass. U.K.*, Vol. 16, pp. 707–22; Vol. 20, pp. 147–79 and pp. 595–604; Vol. 21, pp. 679–86; Vol. 22, pp. 493–500; Vol. 23, pp. 381–6; Vol. 24, pp. 265–70; Vol. 26, pp. 605–8.

SIMPSON, A. C., 1951. The fecundity of the Plaice. *Fish. Invest. Lond.*, Ser. 2, Vol. 17, No. 5, 27 pp.

APPENDIX 1

TABLE 5. THE LENGTH, WEIGHT, AGE AND EGG COUNTS OF FEMALE LONG ROUGH DABS

Fish no.	Total length (cm)	Weight (g)	Age (years)	Egg count 1	Egg count 2	Egg count 3	Egg count 4	Fecundity estimates
				16 March 1955				
2017	25·5	98·0	4	583	646	704	603	126,800
2019	20·0	52·5	4	356	330	358	384	71,400
2020	20·5	51·5	4	313	301	271	305	59,500
2021	20·5	55·0	4	396	395	405	321	75,850
2022	21·0	46·5	4	207	225	191	234	42,850
2039	24·5	86·5	5	273	288	283	277	56,050
2040	17·0	26·5	3	142	186	171	177	33,800
2075	22·0	63·5	4	604	574	605	514	114,850
2076	19·5	36·5	5	243	189	271	235	46,900
2077	19·5	36·0	4	343	326	346	332	67,350
2082	23·0	72·5	5	448	469	367	384	83,400
2083	19·5	41·5	4	190	223	199	203	40,750
				22 February 1956				
1	17·0	25·5	3	158	204	157	158	33,850
2	18·0	33·5	3	231	275	234	186	46,300
3	19·0	38·5	2	241	250	239	234	48,200
4	19·5	44·5	3	289	316	296	279	59,000
5	20·5	58·5	3	376	315	372	340	70,150
6	22·0	76·0	3	429	449	440	396	85,700
7	22·0	70·5	4	392	349	368	408	75,850
8	23·5	87·0	3	589	601	565	577	116,600
9	23·5	96·0	3	716	709	704	759	144,400
10	25·5	103·0	4	629	676	721	636	133,100
11	26·0	110·0	4	760	790	694	835	153,950
12	27·5	151·0	5	958	906	850	842	177,800
13	23·0	77·0	4	362	438	482	368	82,500
14	22·5	68·5	5	518	421	516	478	96,650
15	22·5	67·5	4	328	308	346	252	61,700
16	20·5	48·0	4	349	383	383	470	79,250
17	20·5	45·0	4	270	304	270	315	57,950
18	24·0	81·0	5	396	350	329	428	75,150
19	24·5	92·0	4	424	394	524	452	89,700
20	24·0	94·0	3	586	657	594	539	118,800
21	25·0	117·0	4	927	939	1089	922	193,850
22	25·5	113·5	3	411	427	437	545	91,000
23	26·0	131·0	4	896	762	806	943	170,350

APPENDIX 2

In the analysis of the data the values of fecundity and length were transformed to their logarithms in order to produce a linear relation and to use the standard methods of regression analysis. An interesting point which emerges from the transformation of Simpson's North Sea Southern Bight data is that the geometric mean fecundity is larger for 1947/48, whereas the arithmetic mean fecundity is greater for the 1948/49 winter. The figures are summarized in Table 6. Had the differences between the means for the two seasons been significant, it might have appeared from the analysis that this was a decrease and not an increase. The reason for this anomaly can be seen from the relation between the arithmetic and geometric means for a normal distribution which has been given by Bagenal (1955) and may be written

$$\text{G.M.} = \text{A.M.}/(1 + \sigma^2/\text{A.M.}^2)^{\frac{1}{2}},$$

where A.M. and G.M. are the arithmetic and geometric means and σ^2 is the variance. This gives for 1947/48 calculated G.M. = 72,458; for 1948/49 calculated G.M. = 67,126. The discrepancy between the actual and calculated geometric means is probably due to the data not being normally distributed, which is shown by the difference between the range and 6σ (Table 6).

TABLE 6. SUMMARY OF FECUNDITY STATISTICS FOR SOUTHERN BIGHT PLAICE

	1947/48	1948/49
Arithmetic mean fecundity	84,030	87,740
Variance (σ^2)	2,439,500,000	6,764,600,000
Range	332,000	280,000
6σ	296,316	493,500
Mean log fecundity	4·8317	4·7916
Geometric mean	67,874	61,888

It is clear that the difference between the ranked order of the arithmetic and geometric means of the two sets of plaice fecundity data is due to the large difference between the variances.

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THE ADAPTATIONS OF *LASAEA RUBRA* (MONTAGU), A SMALL INTERTIDAL LAMELLIBRANCH

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(Text-figs. 1–8)

Lasaea rubra is very abundant at Plymouth and has a wide intertidal distribution. It is of small enough size for experiments in which large numbers of animals are needed, and it has recently been studied from several points of view by various workers. Glynne Williams & Hobart (1952) and Morton (1954) have dealt with its ecology, Oldfield (1955) has given a detailed account of its functional morphology, Ballantine & Morton (1956) have examined its feeding and filtering, and Morton (1956) has studied its digestive cycle. Much information on its distribution has been given by Fischer (1940).

Life at the level reached by the high spring tide must offer a hard challenge to a bivalve that feeds and respires by filtering. In the present work a study has been made of the ecology of *L. rubra* in different microhabitats, and experiments have been carried out which shed light on its adaptations to conditions of brief submersion on the upper shore.

INTERTIDAL DISTRIBUTION AND ECOLOGY

L. rubra is found between the levels of the mean high-water spring and low-water spring tides. After a survey had been made of the Plymouth area, the rocks in front of the Plymouth Laboratory at the south side of the municipal swimming pool, and Church Reef, Wembury, were selected as two localities giving a representative range of typical habitats. Fig. 1 shows a simplified diagram of a levelled part of the shore at both places, together with curves, adapted from Colman (1933), showing percentage of exposure at various levels. *L. rubra* typically avoids extremes of high temperature and low humidity by sheltering in shallow crevices, in empty barnacles or in tufts of the lichen *Pygmaea pumila*. In this lichen Colman (1940) found its numbers to reach 12,140 per 100 g of lichen. A dozen or so animals, minute young as well as adults, may cluster together, attaching to the substratum or to each other by temporary byssus threads. The species shows a thigmotaxis that must enable it to avoid exposed positions. When placed in sea water the animals creep actively towards points of attachment, or, if no other substrate is available, aggregate with each other.

Table 1 shows the types of habitat occupied by *Lasaea rubra* at Wembury and at Plymouth, and indicates the composition of the 'community'. Columns 5 and 6 of the table describe the colour and the nature of the algal infection of the shells; the shells of animals from each habitat usually have such characteristic appearance that the source may be easily recognized.

The histograms in Fig. 1 illustrate that there are also characteristic size differences between populations from different habitats. The largest modal and maximum sizes were found at Wembury Low and at Wembury High Spring. As already mentioned by Ballantine & Morton (1956), the animals found at Wembury High Spring are, in fact, larger than those from Wembury High Neap, and special attention has been given to them in this study. The smallest mean size was found in animals from Wembury *Pygmaea*, and Plymouth Medium High and Plymouth Low Neap animals are only a little larger.

The habitat between and inside the empty shells of *Chthamalus stellatus* shelters a characteristic fauna both at Plymouth (see Fig. 2) and at Wembury. There is a 'marine element' consisting of four main species, in the following order of numbers at middle *Chthamalus* level: *Lasaea rubra*, the isopod *Campecopea hirsuta*, and the periwinkles *Littorina neritoides* and young *L. rudis*. Towards the upper barnacle limit *L. neritoides* progressively outstrips the others until at the highest level it alone remains. A second faunal element consists of arthropods of land derivation: the most abundant is the small blue-black collembolan *Anurida maritima*, and, much less frequent, the beetle *Micralymma marinum*. The bright red mite *Bdella interrupta* is occasionally found in large numbers and, usually as single individuals, the pseudoscorpion *Neobisium maritimum*. The primitive marine pulmonate *Otina otis*, the Wembury form of which has been described by Morton (1954), probably also belongs to this terrestrial element and is frequently found in groups of about half a dozen among *Chthamalus* bases.

Legend to Fig. 1

Fig. 1. Schematic diagrams of shore profiles at Church Reef, Wembury, and the Plymouth foreshore, showing the various habitats mentioned in Table 1 in relation to tidal levels. At the left are superposed curves from Colman (1933), showing the average percentage of exposure over a fortnightly tidal cycle. The upper curve is that for splash, and the lower one for total submersion. On the right are shown tidal curves for a single spring tide at Plymouth on 4 September 1956, the upper curve for splash, and the lower one for total submersion. At the top of the diagram are shown the histograms for size distribution (shell length in mm), of the populations of *Lasaea rubra* at the different habitats (August 1956). (1) Wembury High Spring; (3) Wembury *Pygmaea*; (4) Wembury Low Neap; (5) Plymouth High Neap; (6) Plymouth Low Neap.

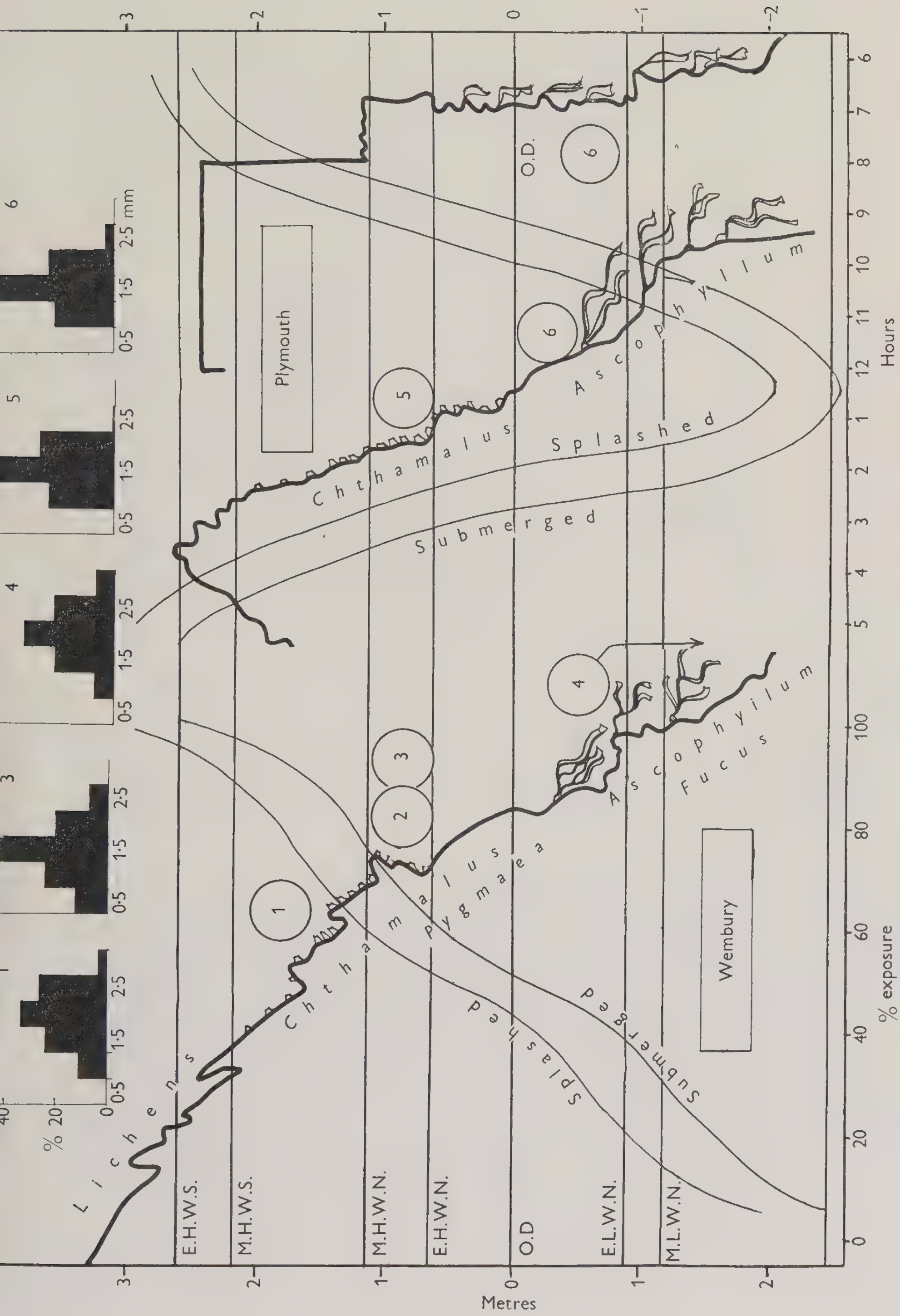
Plymouth
Wembury
E.H.W.S.
M.H.W.S.
M.H.W.N.
E.H.W.N.
O.D
O.D.
E.L.W.N.
M.L.W.N.
Lichens
Chthamalus
Chthamalus
Pygmaea
Ascophyllum
Ascophyllum
Fucus
Splashed
Submerged
Splashed
Submerged
1
2
3
4
5
6
6
% 20
0
0·5
1·5
2·5
2·5 mm
Metres
3
2
1
0
1
2
Hours
6
7
8
9
10
11
12
1
2
3
4
5
% exposure
0
20
40
60
80
100

Fig. 1

TABLE 1. HABITATS OF *LASAEA RUBRA* AT PLYMOUTH AND WEMBURY

Habitat	Description	Percentage of exposure over fortnightly tidal cycle (Colman, 1933). Splash figures in parentheses	Modal length of shells (mm)	Colour of shells	Algal infection of shells	Associated animals
Wembury High Spring	In crevices especially and among *Chthamalus stellatus*, slightly below M.H.W.S.T.	90–95 (75–80)	2·0	Red-brown	Little infection	*Littorina neritoides*, *L. rudis*, *Ligia oceanica*
Wembury High Neap	Among *C. stellatus*, also in crevices, around M.H.W.N.T.	62–75 (50–58)	1·5	Red-brown	Heavy infection—shells eroded with dense filamentous growth—mainly *Entocladia* sp.?	*Campecopea hirsuta*, *Littorina neritoides*, *L. rudis*, *Lipura maritima*
Wembury *Pygmaea*	In tufts of *Pygmaea pumila*, M.H.W.N.T. to E.H.W.N.T.	62–75 (50–58)	1·5	Red-brown	As High Neap	*Campecopea hirsuta*
Wembury Low Neap	In crevices at end of reef below E.L.W.N.T.	18–30 (8–15)	2·0	Pinkish-white to colourless	Little erosion—mainly encrusting forms	*Otina otis* (higher level) *Leucophytia bidentata* *Cingula cingillus* *Hydrogamasus salinus* *Aepus robinii* *Aepophilus bonnairei*
Plymouth High Neap	Among *Chthamalus stellatus*, also in crevices, around M.L.W.N.T.	62–75 (50–58)	1·5	Red-brown	On larger shells—some erosion by filamentous growth of *Entocladia* sp.?	*Campecopea hirsuta* *Littorina neritoides* *Lipura maritima* *Micralymma marinum* *Neobisium maritimum* *Otina otis*
Plymouth Mussel Byssus	In *Mytilus* byssus around and just below E.H.W.N.T.	45–50 (35–40)	—	Paler red-brown	—	—
Plymouth Low Neap	In crevices in artificial concrete-limestone conglomerate, around and slightly above E.L.W.N.T.	30–40 (25–30)	1·5	Colourless or brown encrusted	Shells rather clean, sometimes a few filamentous Cyanophyceae	*Leucophytia bidentata* *Balanus perforatus* *Cingula cingillus* *Turtonia minuta*

The lowest habitat of *Lasaea rubra* on the Plymouth foreshore (Table 1) is formed by chips of limestone cemented together at the base of a concrete platform. Here, *Balanus perforatus* and *Elminius modestus* are the predominant barnacles, and provide abundant cover for large numbers of pale-coloured *Lasaea rubra*. Chocolate-brown patches of the alga *Ralfsia* spread over the whole bare surface of the rock and may encrust some of the larger *Lasaea* shells.

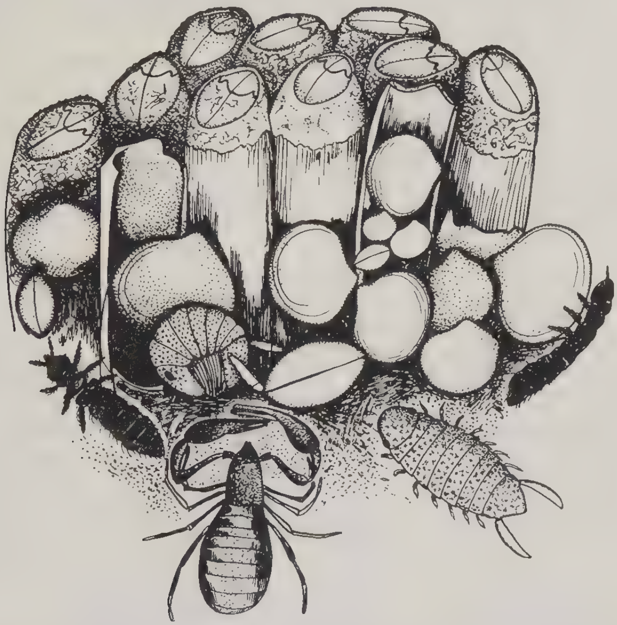

Fig. 2. A group of *Lasaea rubra* from H.W.N.T. at Plymouth, showing their typical situation between the bases of *Chthamalus stellatus*. The associated species, *Campecopea hirsuta* (isopod), *Lipura maritima* (collembolan), and *Neobisium maritimum* (pseudoscorpion) are also shown.

The small lamellibranch *Turtonia minuta* was also found in these habitats. This observation is of interest because the species has not previously been reported as a member of the Plymouth fauna. Although its habits are similar to those of the lowest occurring *Lasaea*, *Turtonia* seems to be confined to this low level and thereby differs from *Lasaea* which is also found farther up the shore. It is smaller than adult *Lasaea* and is pale yellowish white in colour, with darkened umbones. Superficially it is somewhat like *Lasaea*, but careful examination shows that it has quite a different shape, the height being only half the length, whereas it is three quarters of the length in *Lasaea*. Oldfield (1955) has presented a full account of the structure and habits of this bivalve as it occurs at Cullercoats, Northumberland.

PROBLEMS OF SHORT SUBMERSION AT HIGHER LEVELS

It has been calculated that *L. rubra* at its upper limit on Church Reef, Wembury (M.H.W.S.T.), receives an average of only 1 h submersion in 12, and at neap tides may not be totally submerged during a period of 12 consecutive days. Comparable figures for the highest *L. rubra* on Plymouth foreshore are 2·5 h and 7 days respectively. By contrast, *L. rubra* at M.L.W.N.T. receives an average of only 4 h exposure in 12 and is never continuously exposed for more than one tide. At these points the substratum is seldom, if ever, dry and its temperature is low and relatively constant. As has been shown, animals at the highest levels closely compare in size and numbers with those lower down, and the species is indeed typically regarded by collectors as a mid-to-upper tidal animal. Accordingly, the question of how *L. rubra* adapts itself to the harsh conditions of existence high up the shore was thought to be worth investigating and the following experiments were, therefore, carried out.

DIFFERENCES IN FILTERING BEHAVIOUR AT DIFFERENT LEVELS

The filtering rates of Wembury Low Neap and Wembury High Spring *Lasaea* were determined in a total of 48 experiments involving 480 animals: 6 further experiments were carried out with Wembury *Pygmaea* animals. The method used was that described by Ballantine & Morton (1956), a Harvey light absorptiometer being used to measure the decrease in optical density of an Erdschreiber culture of the diatom *Phaeodactylum tricornutum*. Each vessel contained 7·5 c.c. of the culture and 10 large animals (*ca.* 2 mm in length) which were allowed to filter for 1·5 h.

For comparative purposes the results of individual experiments are most conveniently expressed in units of optical density; conversion into volumes of culture filtered, assuming complete filtration of suspended matter, follows the relationship explained in the previous paper.

Fig. 3 shows the value of optical density at intervals of 0·5, 1·0 and 1·5 h for each experiment, and compares the results obtained from studies using Wembury High Spring and Wembury Low Neap *Lasaea*. For both series of experiments solid diagrams are also given, which express the results of experiments which gave the lowest, mean and highest values observed. These results confirm the difference first found by Ballantine & Morton (1956) in preliminary experiments on filtering rates. In general, high-tidal animals show, from the beginning of each experiment, a high and uniformly sustained rate of filtering over 1·5 h. Low-tidal animals show a very low filtering rate for the first hour, but after this time the rate gradually increases until it closely compares with that of the high-tidal animals. With Wembury High Spring animals Ballantine & Morton previously found that the filtering rate decreased after an experimental period of 3 h and became less than that of Wembury Low

Neap animals. The total volumes filtered by the two sets of animals over 6 h were thus approximately the same. Obviously, however, the quicker speed of response and initially more rapid filtering rate of high-level *Lasaea* must be a great advantage to these animals which are covered with water for little more than 1 h at any one time.

The filtering behaviour of animals from Wembury *Pygmaea* (6 experiments) and of others from Plymouth Low Neap was similar to that of the Wembury High Spring animals.

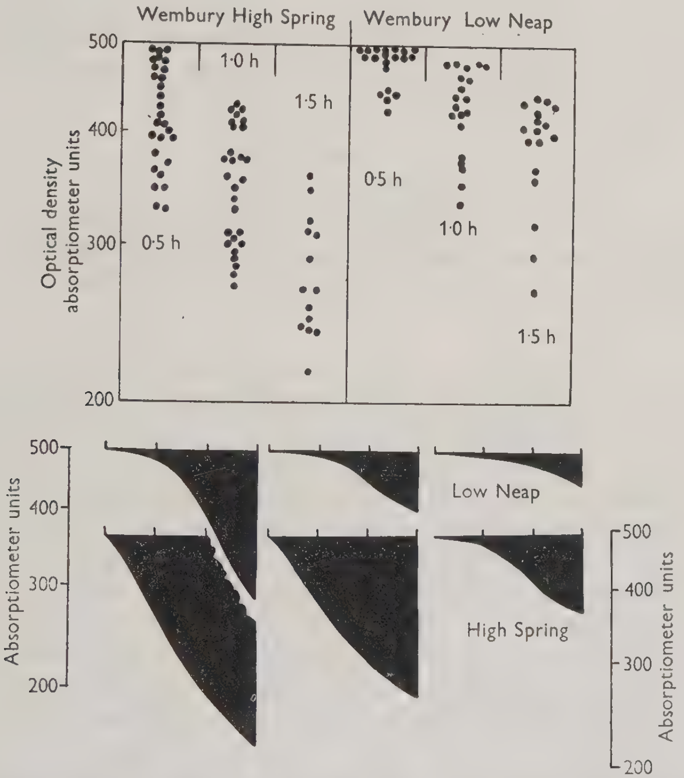

Fig. 3. Results of experiments on the filtering rate of *Lasaea rubra* from Wembury High Spring and Wembury Low Neap. In each experiment, 10 *Lasaea rubra* were placed in 3·75 c.c. of a culture of *Phaeodactylum tricornutum*, and allowed to filter for 1·5 h at room temperature (July 1956). Values are expressed in arbitrary absorptiometer units, and indicate the rate of clearing of suspended matter. *Upper half*: scatter of end values at 0·5, 1·0 and 1·5 h of all experiments (left, Wembury High Spring; right, Wembury Low Neap). *Lower half*: solid graphs from the results of experiments giving the highest, the mean and the lowest values obtained, with animals from Wembury High Spring (top row) and Wembury Low Neap (bottom row).

RESPONSE TO WAVE SPLASH

A further factor redressing the difference in filtering times is wave splash, which prolongs the effective period of submersion (Colman, 1933). The curves in Fig. 1 (right) show the results of 12 h observations of tidal level on the Plymouth foreshore on a day of slight offshore wind. The return of the tide at the *Chthamalus* level is hastened by about 1–1½ h by the action of splash and wave surge. The effective uncovering of a given spot is similarly delayed by splash and even longer by sea water lodged in the interstices of barnacles and in small crevices. On a day of onshore wind and considerable wave surge, the *Chthamalus* zone is hardly dry at all between tides, and the strong preference of *Lasaea rubra* for wave-exposed sites will increase its opportunities to take advantage of splash.

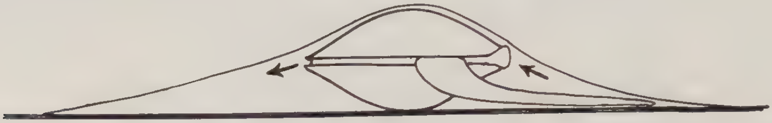

Fig. 4. *Lasaea rubra* as it appears when covered by a water film, with the foot extended and the inhalant siphon (at the anterior end) partly visible. Filtering has begun.

L. rubra is small enough to continue filtering in a layer of water hardly deep enough to cover the whole shell. From observation of animals placed in a film of sea water on a glass slide, as well as of those collected from crevices wet with splash, the valves could be seen to gape and the foot to emerge. After a short time the siphon was extended, and—as could be seen from the movement of particles—filtering began as soon as the shell opened. *L. rubra* is very active under these conditions. The foot can be extended into an extremely thin water film on a flat surface and the animal can pull itself into a position where the water layer is deeper.

In order to take advantage of splash the animal must quickly respond to the presence of water, especially in the hour before the return or after the withdrawal of the tide, when intermittent wetting may last only a few minutes. Experiments were carried out with *L. rubra* which was collected dry, then immediately placed on a glass slide and covered with a film of sea water.

Fig. 5 shows a comparison of the rates of response of Plymouth High Neap and Plymouth Low Neap animals, as estimated from the times needed for the feet to emerge from the shells and for filtering to begin. In 3–4 min each animal in a sample containing 100 was moving about freely with foot and siphon fully extended.

When treated in this way Plymouth High Neap animals were found to respond significantly faster than those from Plymouth Low Neap. Fig. 5 (below, right) indicates the scatter of values for experiments in which about 60 animals were used and in which the numbers filtering were recorded after

20, 40 80, and 120 sec. Cumulative histograms are also given which show the highest, the lowest and the mean values recorded after 2 min in experiments with animals from both levels. The results of typical experiments in which animals were totally submerged in a larger volume of sea water are included in Fig. 5 (upper row) for the purposes of comparison. Fully submerged *L. rubra* show a rate of response which is faster, but only slightly so, than that of animals covered with water on a slide. Further, the difference between High Neap and Low Neap values is greater in the experiments on slides. Although the power to respond quickly is especially well developed in *Lasaea* living at high-tidal levels, it is evidently an intrinsic feature of low-tidal animals as well.

Values for the rate of response of Plymouth High Neap *L. rubra* were plotted against the time for which the animals had previously been exposed on the shore, over the range 0·5–12 h. No obvious correlation was found. *L. rubra* is evidently able to seize any chance provided by splash or other moisture for filtering purposes, and it is probably advantageous that this power of response is highly developed at all times and independent of previous external conditions.

Relation of response rate to salinity

Fig. 5 shows percentage values for response to wetting over a period of 5 min for Plymouth High Neap *L. rubra*, in sea water of salinities ranging from 0·25 to 1·75 normal. A good tolerance, 75% or more of the normal response rate, was found at salinities between 0·75 and 1·25, within which range the animals appeared to behave normally. The ability to maintain its response to wetting in sea-water samples of widely different salinities may be of considerable importance to an animal which must make use, on the one hand of sea water diluted by rain between tides, and on the other, of sea water in which the salt content has risen by evaporation.

Comparative values are shown in Fig. 5 (lower right) for response by Plymouth High Neap and Plymouth Low Neap *L. rubra* to sea water of 0·5 normal salinity. The tolerance of half salinity appears to be significantly greater in the high-level animals. This may be partly because the animals at low-tidal levels are less likely to encounter large differences in salinity. A parallel is found in the recent report by Arnold (1957) of a higher response by high-tidal *Patella vulgata* to stimulus by water of abnormally high or low salinity. With *Lasaea rubra*, however, the difference may in part be caused by the generally slower response of low-tidal animals, as shown in the experiments described on p. 392.

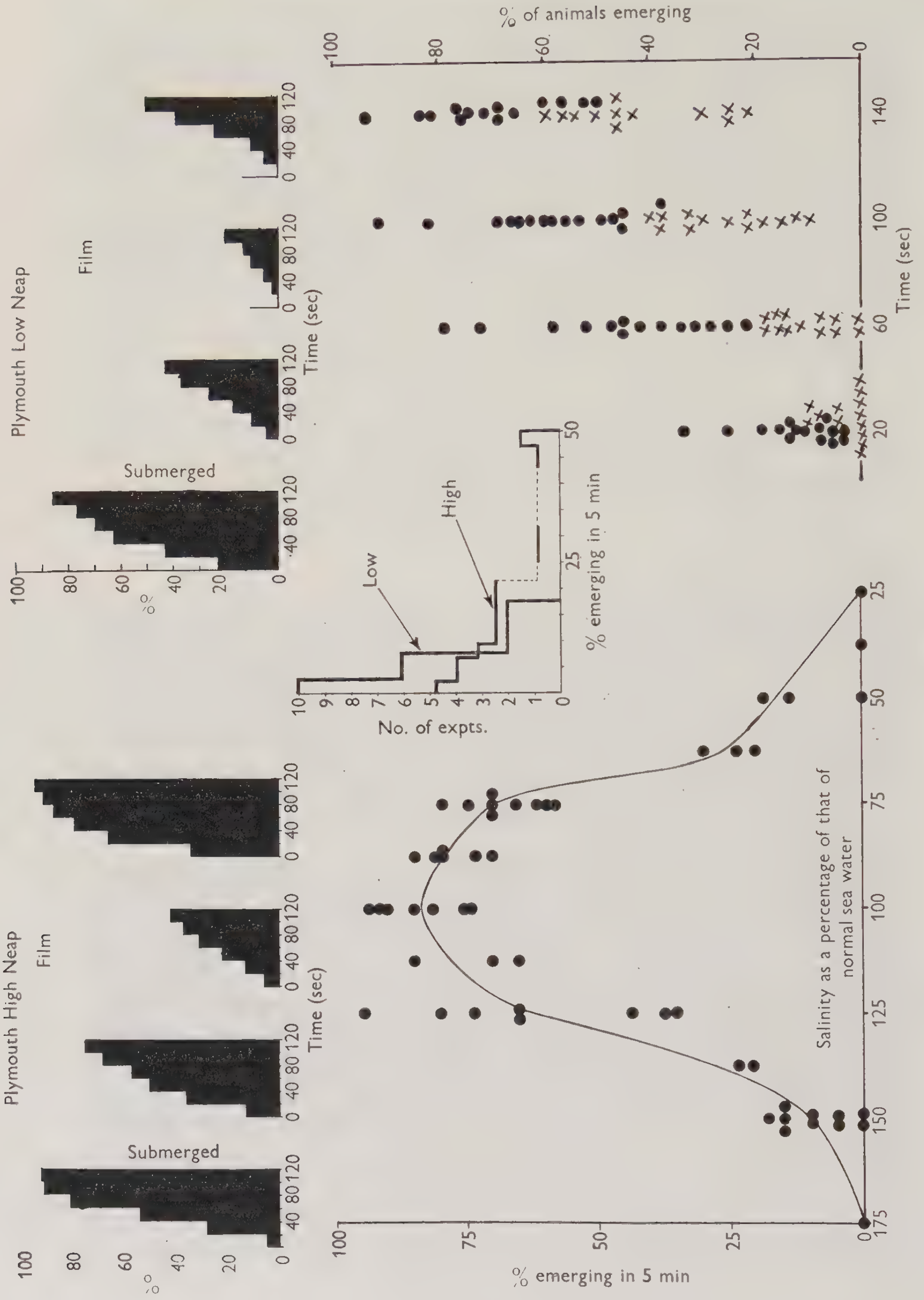
Plymouth Low Neap
Submerged
Film
%
100
80
60
40
20
0
0 40 80 120
Time (sec)
Plymouth High Neap
Submerged
Film
% of animals emerging
Time (sec)
20
60
100
140
Low
High
No. of expts.
% emerging in 5 min
0
25
50
Salinity as a percentage of that of normal sea water
175
150
125
100
75
50
25
% emerging in 5 min

Fig. 5

RESPIRATORY RATES OF HIGH- AND LOW-TIDAL ANIMALS

Method

Animals from Plymouth High Neap, Wembury High Spring and Wembury Low Neap levels were immersed in sea water for 6 h before measurements of respiratory rates were made. Oxygen uptakes were then followed manometrically at 25° C by the direct method of Warburg (Dixon, 1943) and the gas phase used was air. Approximately 75 animals were placed in 3 ml. of sea water in the main compartment of each flask and 0·2 ml. of 20% KOH, together with a roll of starch-free filter-paper, was placed in the centre well. Oxygen uptake was found to be independent of shaking rate in the range 50/100 oscillations/min (5 cm traverse), and there was no evidence of damage when animals were inspected at the end of each experiment. Most measurements of oxygen uptake were continued for 8 h, during which time an approximately linear rate was observed (see Fig. 6). Whitaker (1933) reports that when sea water is used as a manometric medium, the pH may increase from 8·0 to 9·5 as CO_2 is removed by the KOH in the centre well. Robbie (1946), using grasshopper eggs, concludes that the respiring material must offer a rate of CO_2-production of approximately 6μl./h in order to prevent an excessive shift in pH in an unbuffered sea-water medium. In the present work it was found that the sea water pH decreased slightly (usually from 8·0 to 7·6) during the 8 h experimental period, and this was so even when CO_2 production was considerably less than 6μl./h. Gas exchange alone would not account for this effect. Possibly the animals changed the pH of the medium by excreting substances that were slightly acidic to sea water.

After determinations of oxygen uptake had been made, the animals were left for 12 h in alcohol strongly acidified (HCl) to fix the tissues and remove the shells. They were then dried to constant weight at 100° C. The wet weight of an average sample of 100 specimens was about 120 mg, and the dry weight without the shell about 10 mg. The total nitrogen content of the

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Legend to Fig. 5

Fig. 5. Results of experiments on the rate of response of *Lasaea rubra* from Plymouth High Neap and Plymouth Low Neap to splash-wetting (August and September 1956). *Above.* Cumulative histograms showing the response time, by protrusion of the foot, of *L. rubra* barely covered by a film of sea water on a glass slide. In each experiment, 100 animals were used, and observation continued for 2 min. Left: Plymouth High Neap; right: Plymouth Low Neap. In each group of histograms, the mean, lowest and highest result is represented. At the left of the group is shown, for comparison, a histogram for response time, after total submersion in sea water. *Below.* Left: a graph showing the relation between the speed of response (percentage response in 5 min) and the concentration of sea water, ranging from 0·5 to 1·5 normal. Animals from Wembury High Neap. Right: scatter of end values after 20, 60 and 100 sec, of experiments on response rate in sea-water films on slides. ●, Plymouth High Neap; ×, Plymouth Low Neap. *Centre.* Comparison of response rate in a film of sea water of 0·5 normal salinity, by animals from Plymouth High Neap and Plymouth Low Neap.

dried animals was determined by the micro-Kjeldahl method and was found to account for 3·36 and 3·30% of the shell-free dry weight of high and of low animals respectively. All oxygen uptakes were then expressed as Q_{O_2} (N), i.e. μl. O_2/h/mg tissue-N.

Preliminary experiments showed that the rate of oxygen uptake of animals at all levels varied inversely with the number of animals used in the experiment. Thus, high-level samples over the range 2·5–20 mg dry weight gave Q_{O_2} values between 2·5 and 1·2 μl. O_2/h/mg dry wt. respectively: the corresponding values for low-level animals over the same range of biomass were between 1·3 and 0·45 μl. O_2/h/mg dry wt. The influence of the quantity of animals on the rate of oxygen uptake was probably related to the tendency of *L. rubra* to aggregate, thereby reducing the free exchange of respiratory gases. Shaking the flasks very vigorously (*ca.* 120 oscillations/min) did not prevent aggregation, and accordingly, in order to carry out an adequate comparison of respiratory rates, it was necessary to use in all experiments the same narrow range of biomass. It was found that quantities of animals from each level yielding a dry weight of 5–10 mg gave within each group a very comparable level of results. From each tidal level studied, some 20 or more determinations were made, using approximately 1500 animals in each group of experiments.

Results

From the findings summarized in Fig. 6 it will be seen that there is a considerable difference in the rates of oxygen uptake of the medium- and low-level animals, and a smaller but significant difference between the rates of animals from medium high and high levels. Mean Q_{O_2} values are shown in Table 2 and indicate that animals from the low level possess a rate of respiration approximately 50% of that shown by those from the high level.

TABLE 2. COMPARISON OF THE FILTERING AND RESPIRATORY RATES OF *LASAEA RUBRA* WITH THOSE OF *MYTILUS EDULIS*

Mean Q_{O_2} (N) values (μl. O_2/h/mg tissue-N)		Filtering rate (ml./h/mg tissue-N)	
Lasaea rubra		*Lasaea rubra*	
Wembury High Spring	41·4	Value for 10 animals 2 mm in length deduced from data of Ballantine & Morton (1956)	53 (av.)
Plymouth High Neap	35·1		
Plymouth Low Neap	21·6		
Mytilus edulis		*Mytilus edulis*	
Value deduced from data of Jørgensen (1952) for animals 1·5 mm long	29·4	Animals 1·5 mm long	80 (av.)
		Animals 2·9–3·2 mm long (from Jørgensen, 1949)	34 (av.)

Using the results of a recent study by Jørgensen (1952) it has been possible to deduce Q_{O_2} (N) values for *Mytilus edulis* over a wide range of body size. The value corresponding to newly metamorphosed animals of the same size as the *Lasaea* used in the present work (*ca.* 1·5 mm) is included in Table 2 and shows that the respiration rates of the two species are very similar. A similar parallel between the filtering rates of larger *Mytilus edulis* (1·5–2·9 cm length) was found by Jørgensen (1949), and Ballantine & Morton's findings for *Lasaea rubra* (1956).

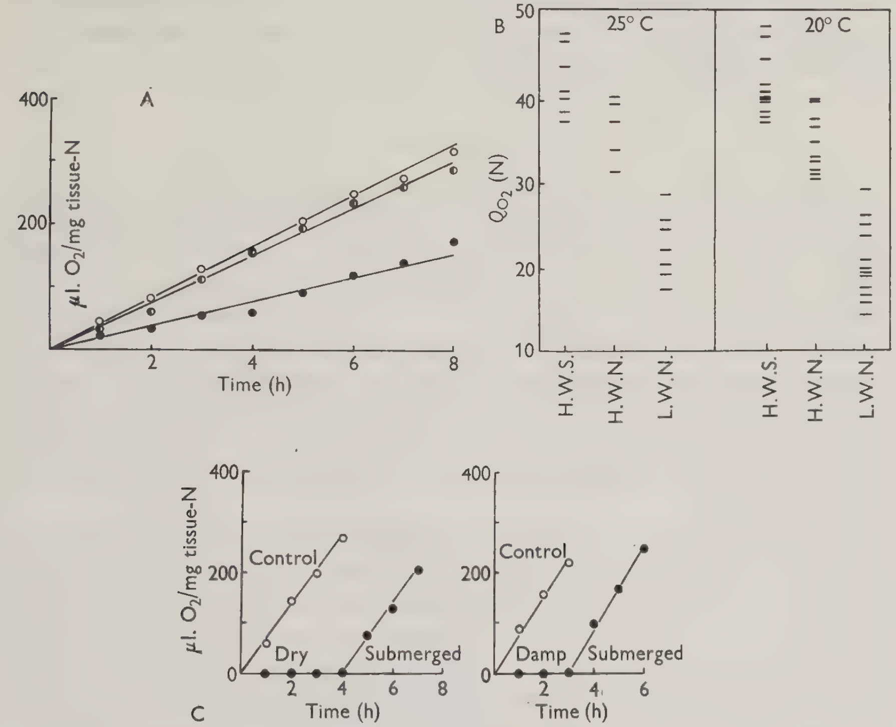

Fig. 6. (A) Respiration of High Spring (○–○), High Neap (◐–◐), and Low Neap (●–●) *Lasaea rubra*. Each point is a mean value of six determinations made at 20° C using 5–10 mg shell-less dry weight of animals. (B) Scatter of Q_{O_2} (N) values observed for *L. rubra* from three tidal levels. Left: experiments carried out at 25° C with 8–10 mg shell-less dry weight of animals for each determination. Right: experiments, conducted at 20° C, in each of which 5–10 mg shell-less dry weight of animals was used. (C) Respiration of High Spring *L. rubra* when dry (left), damp (right) and when submerged in sea water. ○–○, Animals submerged at once; ●–●, animals submerged after 4 h in a dry state (left) or 3 h in the damp state (right). Each point is the mean of four determinations carried out at 25° C with 5 mg shell-less dry weight of animals.

Respiration of 'dry' and 'wet' animals in moist air

Approximately 40 *L. rubra* from Wembury High Spring level (*ca.* 3 mg dry wt.) were placed, after careful drying of the shells, in Warburg flasks equipped with two side-arms. In each side-arm was placed 1·5 ml. of sea water. To each of three other flasks the same number of animals was added, immersed as usual in 3 ml. of sea water. Oxygen uptakes of dry and submerged animals were then measured for 3 h, after which time the water from the side-arms was tipped upon the dry animals, and measurements of oxygen consumption continued for a further 3 h. A second experiment was made in which, instead of 'dry' *L. rubra*, the animals used lay in a water film insufficient to immerse the whole shell. As may be seen from Fig. 6, there was no detectable oxygen uptake by either 'dry' or 'wet' animals in moist air. Respiration was resumed at the normal rate, however, when the animals were fully immersed in sea water.

RESISTANCE TO DESICCATION

The low-tidal habitats of *L. rubra* are nearly always damp and are probably never raised to a high temperature by the sun. At higher levels, as may be inferred from the figures given by Morton (1954), conditions of high temperature and low humidity must on hot days offer a greater problem. Tufts of *Pygmaea pumila* must at times present very harsh conditions, for although in cooler weather or on wet days they remain moist between tides, they may in summer become hard and brittle at midday low tides. The shells of *Lasaea rubra* sheltering within them are perfectly dry. Similarly, with Wembury High Spring and Plymouth High Neap localities, the habitat warms up and dries out through insolation of the surrounding rock mass, and the shells of *Lasaea* become dry.

Animals from each of the four locations Wembury High Spring, Plymouth High Neap, Plymouth Low Spring and Wembury *Pygmaea* were used in experiments carried out to determine the amount of weight lost by evaporation of water over a period of 12 h exposure to extreme conditions of high temperature and low humidity, i.e. at 30° C in a relative humidity of 0–5%. In other experiments animals from each locality were exposed to these conditions for periods of $1\frac{1}{2}$, 4, 7 and 12 h and their speeds of recovery, and the percentages of the totals which recovered when they were replaced in sea water, were then determined. Recovery was assumed to have occurred the moment the foot or the siphon emerged.

Method

In studies of the rates of water loss, a thermogravimetric method was used in which the change of weight was measured by counterbalancing electromagnetically, as described by Gregg (1955). The balance was fixed to a wooden frame over a bath of water, the temperature of which was regulated by a Sun-Vic thermostatic control linked to an immersion heater. In the bath was placed a chamber (500 c.c.) in which the humidity was controlled by the use of appropriate concentrations of sulphuric acid. A wire gauze reaction vessel was placed in the humidity chamber, and suspended from the underside of a balance pan. In each experiment a sample of about 200 aggregated animals, drained of surplus water and with their shells only slightly wet, was placed in the reaction vessel. The fresh weight was determined in the previously weighed reaction vessel. The humidity chamber was then closed by a divided cork, and weight loss was measured at intervals by briefly removing the cork and equilibrating the reaction vessel against the magnet of the balance.

Fig. 7 shows the curves for water loss over an experimental period of 11 h, calculated from the beginning of the second hour in the drying vessel (it was considered that most of the water loss after the first hour could be accounted for by the removal of water from the surface of the shells and the pallial cavity). It is apparent that even in the high-tidal samples, the shells are unable to remain perfectly sealed against prolonged exposure to dry air, and, in fact, most of the animals replaced in water after more than 3 h drying at first floated with the umbones down. An air bubble could be seen in the pallial cavity, which was released when the animal extended its foot. Water loss from Plymouth Low Neap animals appeared to be about twice that from the

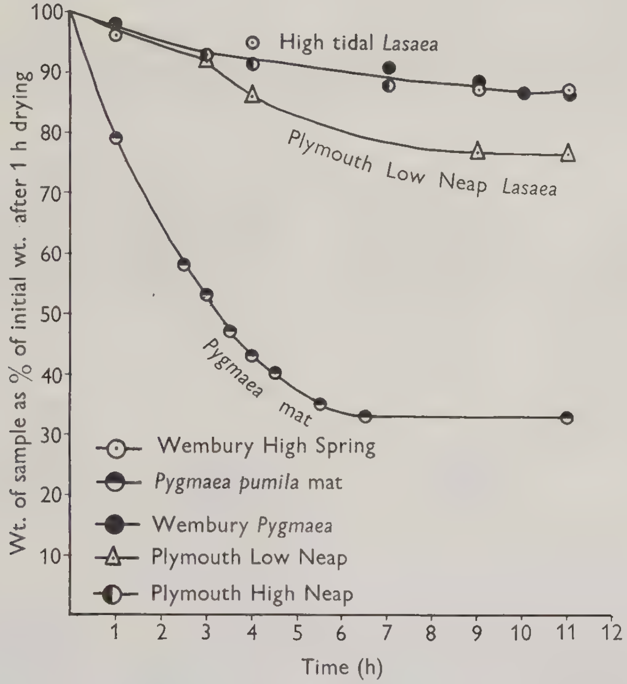

Fig. 7. Graphs showing loss of weight, by evaporation of water, of *Lasaea rubra* from various habitats. A sample of about 200 animals was exposed over a period of 12 h to a temperature of 30° C in an atmosphere of 0–5 % relative humidity. Weights are expressed as percentages of the weight of the sample after the first hour of drying, during which only surface water evaporated. The lower curve shows the values for the loss of weight by the intertidal lichen *P. pumila* under the same experimental conditions.

samples from the other three locations, amounting in 11 h to 23 % by weight. In animals from Plymouth High Neap, Wembury High Spring and Wembury *Pygmaea*, the curves for water loss were almost identical over the 11 h experimental period. Thus, the low-tidal animals appear to be less able to keep their shells tightly closed over a long period. A behavioural difference in Plymouth Low Neap animals could sometimes be noticed which confirmed this result, namely that when these *Lasaea* were left in the dry state they did not close their shells as quickly as did those from higher levels, and occasionally their shells remained open for a considerable time after the animals were removed from sea water.

TABLE 3. RESULTS OF EXPERIMENTS ON RATE OF RECOVERY AND PERCENTAGE OF SURVIVAL AFTER EXPOSURE TO VARIOUS TEMPERATURES AND HUMIDITIES

Habitat	Experimental conditions	Percentage recovery in 15 min after exposure for				Percentage dead after	
		1½ h	4 h	7 h	12 h	7 h	12 h
Wembury High Spring	30° C; dry air	100	95	89	97	0	0
Wembury *Pygmaea*	30° C; dry air	91	55	92	97	0	0
Plymouth High Neap	30° C; dry air	100	82	70	54	0	24
Plymouth Low Neap	30° C; dry air	65	42	31	3	5	50
Plymouth Low Neap	18° C; dry air	—	84	—	—	—	—
Plymouth Low Neap	18° C; 50 % R.H.	—	82	—	—	—	—
Plymouth Low Neap	25° C; dry air	—	67	—	—	—	—
Plymouth Low Neap	25° C; 50 % R.H.	—	64	—	—	—	—
Plymouth Low Neap	30° C; 100 % R.H.	—	92	—	—	—	—
Plymouth Low Neap	30° C; 50 % R.H.	—	78	—	—	—	—

Results of experiments on speed of recovery and percentage survival after periods of exposure of 1½, 4, 7 and 12 h are shown in Table 4. Cumulative histograms are presented in Fig. 8. The most marked differences are seen between Plymouth Low Neap animals on the one hand, and those from Wembury High Spring, Plymouth High Neap and Wembury *Pygmaea* on the other. Thus, low-tidal animals showed after 1½, 4 and 7 h exposure a markedly slower rate of recovery than that of high-level animals when placed in sea water. After 7 h, 5 % of the low-level animals had died, but the remainder eventually recovered. After 12 h exposure, 50 % had died and although the remaining animals recovered they did so at a very slow rate. By contrast, in experiments with Wembury High Spring and Wembury *Pygmaea* animals it was found that even after prolonged exposure (12 h) there was almost complete recovery (80–90 %) within 12 min of immersion in sea water. In experiments with Plymouth High Neap animals it was found that 24 % of the sample were dead after exposure for 12 h and, further, the recovery of the remainder from this, and shorter, periods of exposure was slower than that observed in experiments with Wembury High Spring and Wembury *Pygmaea* animals.

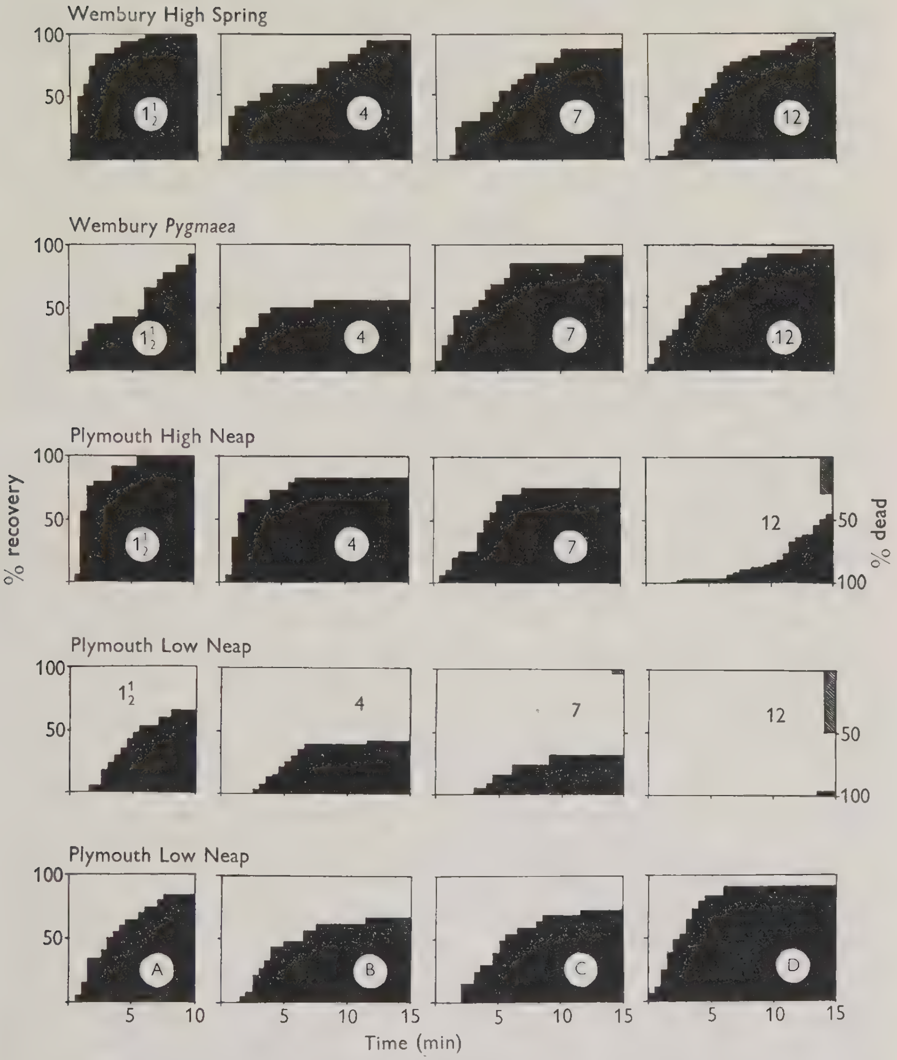

Fig. 8. Cumulative histograms showing the rate of recovery on return to sea water of *Lasaea rubra* which had been exposed to high temperatures and low humidities. Rows 1–4 describe the recovery of animals after exposure to an atmosphere of 0–5 % relative humidity at 30° C. Samples were returned to sea water after 1·5, 4, 7 and 12 h as indicated on each diagram. Row 5 describes the recovery of animals exposed for 4 h to 0–5 % relative humidity at 18° C (A); 50 % relative humidity at 25° C (B); 0–5 % relative humidity at 25° C (C) and 100 % relative humidity at 30° C (D).

Further experiments were carried out in which Plymouth Low Neap animals were exposed for 4 h to dry air at 18 and 25° C, to 50% relative humidity at 25° C and to saturated air at 30° C (see Fig. 8, bottom row). All these sets of conditions were easily withstood, for when the animals were placed in sea water at the end of the exposure period, 60–90% recovered within 15 min and the whole sample eventually recovered in every case.

DISCUSSION

Geographical considerations

Few bivalves can have pushed their intertidal distribution as high as that of *Lasaea rubra* at its upper limit. Moreover, this species appears to flourish as well, and to grow as large at high levels as it does at low, and the higher respiratory rate, and initially faster filtering rate, imply that *L. rubra* maintains a high metabolic activity on the upper shore.

Although information on growth rates at various levels is lacking, consideration of the geographical distribution of *L. rubra* sheds further light upon its occurrence at high levels on the British shore. In Britain *L. rubra* is absent from the eastern English Channel and the southern North Sea. It occurs widely around the western and south-western coasts, including Ireland, the Irish Sea, Scotland and the Western Isles; and enters into the North Sea as far south as Scarborough. In distribution it thus resembles the group of intertidal animals that includes *Osilinus lineatus*, *Paracentrotus lividus*, *Gibbula umbilicalis*, *Chthamalus stellatus* and possibly *Otina otis* (cf. Morton, 1954) regarded by Orton (1920) and by Southward & Crisp (1954) as constituting a 'southern element' in the British fauna. All are summer breeders and, as their distribution outside Britain shows, are here at the northern limit of their range. *Lasaea rubra* is widespread in lower latitudes and, although its equatorial distribution is unknown, it is found generally throughout the North and South Atlantic and extends into the Mediterranean. Chapman (1955) found it abundant in the *Corallina* turf at the Azores. The strong tolerance by *Lasaea rubra* for the relatively high temperatures of the upper shore becomes better understood in the light of its distribution. As with the 'southern' barnacle, *Chthamalus stellatus*, its optimum situations would appear to be where there is a maximum of warmth from the sun, subject always to protection from very prolonged high temperatures.

Adaptations

If, as its geographical distribution suggests, *Lasaea* has a preference for the English upper shore, considerable interest attaches to several adaptations which might help the animal to offset the adverse conditions of brief submersion and shortened filtering and respiring times. Small size is in itself an advantage which must enable the animal to filter from small volumes of water intermittently splashed on to it between tides. In addition, there are behavioural adaptations such as rapid response to wetting—better developed at upper levels in the Plymouth population—which equip *Lasaea* as an 'opportunist' species able to take immediate advantage of splash. Southward (personal conversation) finds the same adaptation in high level *Chthamalus stellatus*. A further adaptation is that of site selection: thigmotaxis, negative phototaxis and preference for moisture all lead *Lasaea rubra* to aggregate in small crevices and in lichen tufts where splash probably collects and where desiccation is less likely. Activity in a rather wide salinity range (see p. 392) may also enable *Lasaea* to take advantage of splash diluted with rain water or to continue filtering after salinity has risen slightly by evaporation.

Our experiments suggest that animals at higher levels possess certain physiological adaptations concerned with filtering and respiration which must also protect them against the rigours of short submersion. Thus, studies of filtering rates disclose that whereas animals from high up the shore begin to filter rapidly as soon as they are wetted, low-tidal animals at first show a much lower filtering rate. After approximately 3 h, however, low-level animals build up a filtering rate approaching that of higher animals. These findings, while not implying an intrinsic difference between the filtering rates of high- and low-level *Lasaea*, do, however, draw attention to the much quicker response of high-tidal animals.

In contrast to the feeding-rate studies, investigation of the respiratory rates of *L. rubra* from Wembury High Spring, Plymouth High Neap and Plymouth Low Neap levels has shown marked intrinsic differences. Thus, animals from high up the shore respire at approximately twice the rate of Low Neap animals. Interest also attaches to the further finding that these high-level animals are unable to respire in moist air when damp or dry; evidently the price of high-tidal existence is paid not by using atmospheric oxygen, but by developing a rapid rate of aquatic respiration during brief periods of submersion.

There seems a need to explain why no respiration could be detected when animals are covered by a thin layer of water, though in this state filtering may be taking place. However, absence of measurable respiration need not imply absence of feeding, because collection of solid particles by the gill—although a ciliary process—must be relatively much faster than gaseous exchange. It is known that *L. rubra* is able to clear all particles from its own volume of water in 2 min. A few minutes' filtering of splash may thus be valuable from the point of view of feeding. The animal ingests large amounts of bottom detritus, such as may be stirred into suspension in falling splash, in addition to the rich phytoplankton found in Plymouth water samples at or near the substratum where *Lasaea* was collected (Ballantine & Morton, 1956).

Experiments on resistance to desiccation and recovery from exposure to high temperatures have shown that Wembury High Spring and Wembury *Pygmaea* animals are well able to withstand conditions of high temperature and low humidity far more unfavourable than any which can occur in the field. In addition, it has been found that whereas Plymouth High Neap animals are only slightly less resistant than the Wembury High Spring and Wembury *Pygmaea Lasaea* to harsh experimental conditions, Plymouth Low Neap animals are much less resistant than the Wembury high-level *Lasaea* in this respect. These differences accord with tidal position (see Fig. 8), and reveal a clear physiological difference between populations at high and low levels. The shell pigmentation may be a relevant factor in temperature resistance, and it is hoped to investigate this further. Resistance to desiccation at high levels must depend upon tight closing of the shell when the animals are dry, and this evidently allows no respiratory exchange with damp air, or even when the shells are covered with a water film. Aerial respiration by a damp body surface must be paid for by restriction to 'safe' habitats of high humidity.

Comparison with other species

Other animals sharing the high-level habitat of *L. rubra* are the barnacle *Chthamalus stellatus* and the small periwinkle *Littorina* (*Melarhaphe*) *neritoides*. In some features of their ecology these bear comparison with *Lasaea rubra*. *Chthamalus stellatus* is also a filter feeder, but appears (see Monterosso, 1928) to be able to respire to some extent in a moist atmosphere. No detailed data are available for comparative respiration at various levels. Southward (1955), in a study of the influence of tidal level on cirral activity of barnacles, finds that whereas *Elminius modestus* and *Balanus balanoides* have a higher rate of cirral beat at low-tidal levels, *Chthamalus stellatus*—except at one locality—maintains an equally high rate at high levels. This feature of *C. stellatus* may offer partial compensation for short submersion time. A further advantage enjoyed by high-level *C. stellatus*—as compared with *Lasaea rubra*—must be its faster method of filtering, by means of cirri, under muscular control. A forthcoming account by Southward of filtering mechanisms of barnacles should make a comparison easier. *Littorina neritoides* depends on a film of moisture for feeding (Lysaght, 1941). It crops small algae and lichens with its radula, and does not need either submersion or heavy splash. Respiration can take place in moist air, although Fischer, Duval & Raffy (1933) have shown that it is five or six times higher in water than in air. Patané (1933) finds that *L. neritoides* regains activity after a few minutes in water, following 5 months' exposure to air.

Both the barnacle and the periwinkle reproduce by liberating free-swimming larvae. *Lasaea rubra* incubates the young between the gill lamellae. A free-swimming stage permits wide distribution and site selection, and such an opportunity for intermixture of extremes of the population and exchange of settling sites seems to be lacking in *L. rubra*. Young animals settle near the parent, and this tendency to isolation of small populations offers a method by which physiological differences within the species—for example, in pigmentation, and in respiratory rate—might lead to ecological subspeciation.

A recent study by Segal, Rao & James (1953) of differences in water propulsion by *Mytilus californianus* at three depths, 'deep water at 30 ft.', 'low intertidal' and 'mid-intertidal', reveals higher activity as depth increases. The authors correlate this finding with temperature adaptation in a poikilothermal animal. But this situation is hardly comparable with that of intertidal *Lasaea rubra*, subjected as it is to long exposure on the upper shore. The lowest population of *L. rubra* corresponds in level to the highest ('mid-intertidal') population of *Mytilus*. The 'mid-intertidal' *Mytilus* are, moreover, smaller than the 'low-intertidal', and these in turn are smaller than the deep ones. It would appear that *M. californianus* is a species restricted as to size and probably metabolism, on entering the intertidal zone from off-shore. *Lasaea rubra*, however, with a wholly intertidal distribution, holds its own well at high water. A relevant fact is that the highest filtering rates recorded were from high-tidal animals at an experimental temperature of 22° C (Ballantine & Morton, 1956).

The mode of life, especially the reproduction and rate of growth, of *Lasaea rubra* invites further study. But from what we already know of its adaptations to life in the upper tidal zone, this bivalve seems to stand out—physiologically as well as geographically—as a member of the rather distinct 'southern component' of our intertidal fauna.

It is a pleasure to express our thanks to Mr E. R. March of Plymouth Technical College for constructing the electromagnetic sorption balance used in some of our experiments. Two of us (J. E. M.) and (A. D. B.) have been using London University Tables at the Plymouth Laboratory, and one of us (E. D. S. C.) is indebted to International Paints Ltd. for a Research Fellowship. We are most grateful to the Director and many members of the staff of the Plymouth Laboratory for the encouragement and helpful advice they have given us throughout the research.

SUMMARY

A study has been made of the ecology of the small intertidal lamellibranch *Lasaea rubra* at various tidal levels at Plymouth and Wembury. In addition, experiments have been carried out to investigate physiological and behavioural differences arising from varying amounts of submersion at different tidal levels, and the following findings have been made.

During the first hour after their submersion by sea water, *L. rubra* from high up the shore filter at a rate approximately twice that of animals which live lower down. After 2 h, however, both sets of animals filter at the same rate.

High-level animals respond significantly faster to wetting by splash and can tolerate a considerable range of salinity. They also respire at a rate

approximately twice that of low-level animals. Respiration, however, is not detectable when the animal is not immersed in sea water.

L. rubra from all levels show a good resistance to desiccation when exposed to high temperature and low humidity, but over a 12 h period low-level animals lose more water by evaporation. Moreover, when the low-level animals are subjected to these harsh experimental conditions they show a lower rate of recovery and a lower percentage of survival.

These findings are discussed with reference to the geographical distribution of *Lasaea rubra* and its status as a warmth-loving member of a southern faunal element.

REFERENCES

Arnold, D. C., 1957. The response of the limpet, *Patella vulgata* L., to waters of different salinities. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 121–8.

Ballantine, D. & Morton, J. E., 1956. Filtering, feeding and digestion in the lamellibranch *Lasaea rubra*. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 241–74.

Chapman, G., 1955. Aspects of the fauna and flora of the Azores. VI. Density of animal life in the *Corallina* alga zones. *Ann. Mag. nat. Hist.*, Vol. 8, pp. 801–5.

Colman, J. S., 1933. The nature of the intertidal zonation of plants and animals. *J. mar. biol. Ass. U.K.*, Vol. 18, pp. 435–76.

—— 1940. On the faunas inhabiting intertidal sea-weeds. *J. mar. biol. Ass. U.K.*, Vol. 24, pp. 129–83.

Dixon, M., 1943. *Manometric methods*. 122 pp. Cambridge University Press.

Fischer, P. H., 1940. Sur l'habitat de *Lasaea rubra*. *Bull. Mus. Hist. nat., Paris*, Ser. 1, T. 12, pp. 433–5.

Fischer, P. H., Duval, M. & Raffy, A., 1933. Études sur les échanges respiratoires des littorines. *Arch. Zool. exp. gén.*, T. 74, pp. 627–34.

Glynne Williams, J. & Hobart, J., 1952. Studies on the crevice fauna of a selected shore in Anglesey. *Proc. zool. Soc. Lond.*, Vol. 122, pp. 797–824.

Gregg, S. J., 1955. An electromagnetic sorption balance. *J. chem. Soc.*, pp. 1438–9.

Jørgensen, C. B., 1949. The rate of feeding by *Mytilus* in different kinds of suspension. *J. mar. biol. Ass. U.K.*, Vol. 28, pp. 333–44.

—— 1952. Efficiency of growth in *Mytilus edulis* and two gastropod veligers. *Nature, Lond.*, Vol. 170, p. 714.

Lysaght, A. M., 1941. The biology and trematode parasites of the gastropod *Littorina neritoides* (L.) on the Plymouth foreshore. *J. mar. biol. Ass. U.K.*, Vol. 25, pp. 41–67.

Monterosso, B., 1928. Studii cirripedologici III. Persistenza dei fenomeni respiratori nei Ctamalini mantenuti in ambiente subaereo. *Boll. Soc. biol. Sper.*, Vol. 3, pp. 1067–70.

Morton, J. E., 1954. The crevice faunas of the upper intertidal zone at Wembury. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 187–224.

—— 1956. The tidal rhythm and action of the digestive system in the lamellibranch *Lasaea rubra*. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 563–86.

Oldfield, E., 1955. Observations on the anatomy and mode of life of *Lasaea rubra* (Montagu) and *Turtonia minuta* (Fabricius). *Proc. malacol. Soc. Lond.*, Vol. 31, pp. 226–49.

Orton, J. H., 1920. Temperature, breeding and distribution in marine animals. *J. mar. biol. Ass. U.K.*, Vol. 12, pp. 339–66.

PATANÉ, L., 1933. Sul comportamento di *Littorina neritoides* L., mantenuta in ambiente subaereo ed in altre condizione sperimentali. *R.C. Accad. Lincei*, Ser. 6, Vol. 17, pp. 961–7.

ROBBIE, W. A., 1946. The quantitative control of cyanide in manometric experimentation. *J. cell. comp. Physiol.*, Vol. 27, pp. 181–209.

SEGAL, E., RAO, K. P. & JAMES, T. W., 1953. Rate of activity as a function of intertidal height within populations of some littoral mollusca. *Nature, Lond.*, Vol. 172, p. 1108.

SOUTHWARD, A. J., 1955. On the behaviour of barnacles. II. The influence of habitat and tidal level on cirral activity. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 423–33.

SOUTHWARD, A. J. & CRISP, D. J., 1954. The distribution of certain intertidal animals around the Irish coast. *Proc. R. Irish Acad.*, Vol. 57 B, pp. 1–29.

WHITAKER, D. M., 1933. On the rate of oxygen consumption by fertilized and unfertilized eggs. IV. *Chaetopterus* and *Arbacia punctulata*. *J. gen. Physiol.*, Vol. 16, pp. 475–95.

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STUDIES IN THE GENUS *FUCUS* L.[1]
I. *FUCUS DISTICHUS* L. EMEND. POWELL

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(Plates I and II and Text-fig. 1)

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INTRODUCTION

Apart from the alga currently known as *Fucus spiralis* L. emend. Batt., and certain hermaphrodite hybrid *Fucus* forms that will be discussed in a later paper, several other hermaphrodite forms of the genus *Fucus*[1] occur on the shores of the North Atlantic, the North Pacific and the Arctic oceans. These forms have been described under many different names by many authors; and there has been and still is much confusion, as well as real difference of opinion, concerning their delimitation and taxonomic status.

Thus, many European authors, including Rosenvinge (1893) for Greenland, Börgesen (1902) for the Faeröes, and Jónsson (1903) for Iceland, regard all such forms, occurring in the various parts of the North Atlantic studied in detail by them, as ecological forms of a single species, and have shown that, at least in some parts of this wide area, all possible intermediate forms between the more distinctive extreme forms can be found. The name *F. inflatus* L. has been generally used by recent European phycologists[1] for this complex of forms and several characteristic forms have been widely recognized as follows:

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[1] The full citation of author's names for this genus is *Fucus* L. (1753) pro parte, emend. Dec'ne et Thur. (1845); for most purposes, however, it is sufficient to shorten the citation to *Fucus* L. (The often used citation '*Fucus* (L.) Dec'ne et Thur.' is nomenclaturally incorrect.)

Rosenvinge (Greenland, 1893)	Börgesen (Faeröes, 1902)	Jónsson (Iceland, 1903)
Fucus inflatus L., M. Vahl	*Fucus inflatus* L., M. Vahl	*Fucus inflatus* L., M. Vahl
var. *edentatus* (De la Pyl.) Rosenv.	f. *edentata* (De la Pyl.) Rosenv.	f. *typica* Jónss. (=*F. edentatus* De la Pyl.)
var. *evanescens* (C. Ag.) Rosenv.	(only a few specimens found that approached f. *evanescens* (C. Ag.) Rosenv.)	f. *evanescens* (C. Ag.) Rosenv.
var. *linearis* (Oed.) Rosenv.	f. *linearis* (Oed.) Rosenv.	f. *linearis* (Huds.)
—	f. *disticha* (L.) Börg.	f. *exposita* Jónss. (=f. *disticha* (L.) Börg.)

The type form, =f. *edentatus* (De la Pyl.), and f. *evanescens* (C. Ag.), of these authors are large plants found on sheltered or semi-exposed shores; f. *linearis* is a smaller, slender plant, usually found in littoral pools; f. *disticha* (L.) Börg., =f. *exposita* Jónss., is a small, narrow, but robust form developed on coasts very exposed to swell and wave-action.

Recent North American authors, on the other hand, interpret certain essentially similar North American Atlantic and Pacific fucoids as distinct species, as follows:

(i) North-east Atlantic coast of North America (Taylor, 1937):
F. edentatus De la Pyl., *F. evanescens* C. Ag., *F. filiformis* Gmel. (in pools), *F. miclonensis* De la Pyl.; (also listed are *F. serratus*, *F. vesiculosus* and *F. spiralis*).

(ii) Pacific coast of North America (Gardner, 1922; Setchell & Gardner, 1925):
F. edentatus De la Pyl., with five forms recognized and described. (Species based upon a Newfoundland plant.)
F. evanescens C. Ag., with twenty-one forms. (Species based on North Pacific material.)
F. furcatus C. Ag., with thirteen forms. (Species based on North Pacific material.)
F. membranaceus Gardner, with six forms.
F. nitens Gardner.

These authors reject the name *F. inflatus* L. altogether, on the grounds that Linnaeus's original short description and the fragmentary specimens bearing the name '*inflatus*' in the Linnaean Herbarium are inadequate to delimit any particular species of *Fucus*.

In order to determine the correct names to apply to two forms of '*Fucus inflatus*' found in Britain it has been necessary, therefore, to consider first the following taxonomic and nomenclatural problems: (i) are the fucoids listed above sufficiently distinct to be regarded as separate species, or are they best regarded as forms of a single extremely variable species; (ii) if best regarded as a single species, is the use of the disputed specific epithet '*inflatus* L.' justifiable; and (iii) is the particular nomenclature adopted for formae by, for example, Rosenvinge, Börgesen and Jónsson, in best accord with modern taxonomic concepts and nomenclatural rules and procedures?

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[1] Lund (1949*a*, *b*) uses the name *Fucus edentatus* De la Pyl. in reporting the recent immigration of this fucoid into Danish waters but, in reply to an inquiry, he has informed me that he considers that the Danish plants should be named *Fucus inflatus* L., f. *edentatus* (De la Pyl.) in accordance with the views of Rosenvinge, Börgesen and others.

Species, subspecies, or formae?

After full consideration of all that has been published on the autecology and world distribution of these forms, I am in agreement with Börgesen (1902), Jónsson (1903), and other authors, that the numerous forms described are not sufficiently distinctive to warrant separate specific status, but are best interpreted for the present as forms of a single, extremely plastic, highly successful and widely distributed species. Chief weight is given to the fact that, at least near the centres of distribution of the species (e.g. in the Faeröe Islands and Iceland, and probably in northern Europe and on the Pacific coast of North America), whole series of forms (clines) of this fucoid intermediate in character between the extreme forms are very common. The close affinity of the various forms is further emphasized by the occurrence of caecostomata (see p. 418) in varying number in most, and probably all, of them, but not in any other species of *Fucus*.

Near the centres of distribution of the species it is possible to interpret the extreme forms as products of their ecological environment, with the intermediate forms developing under intermediate environmental conditions. Towards the southern and northern limits of distribution, however, the species is often represented by populations of only one or two of the more distinctive (best adapted) forms, confined to restricted habitats and often geographically isolated. Such isolated populations often have a very limited range of form, and several could well be regarded as genetically adapted ecotypes. For these reasons the more distinctive forms are interpreted as *subspecies* in the present paper.

THE CASE FOR REJECTION OF THE NAME *FUCUS INFLATUS* L.

The name *F. inflatus* is first used by Linnaeus (1737) in *Flora Lapponica*, Ed. 1, p. 351, No. 468, the material being collected (by Linnaeus?) at Rørstad in Nordlanden, Norway, on his Lapland journey in 1732. It is first described in this way: '*Fucus folio bifido, laciniis ovato-lanceolatis inflatis, ad apicem divisis.*' The diagnosis given by Linnaeus in *Species Plantarum* (1753, p. 1159) is '*Fucus fronde bifida: laciniis ovato-lanceolatis inflatis apice divisis. Fl. lapp.* 468, *Fl. suec.* 1004. *Habitat in Oceano Atlantico*'. Finally, in *Systema Naturae* (1766) and *Systema Vegetabilium* (1774), Linnaeus slightly enlarges the diagnosis to read as follows: '*Fronde plana, dichotoma, integerrima, punctata, ovato-*

lanceolata, inflata, apice diviso'; this may be translated as 'frond plane [flat], dichotomously branched, entire [margins], punctate [presumably this refers to the presence of cryptostomata], ovate-lanceolate [see below], inflated [probably refers to the presence of irregular inflations], divided at the apices'. These diagnoses of *F. inflatus* are certainly inadequate to define any particular species as understood to-day; Linnaeus is presumably describing sterile material and the diagnoses could fit, but could not define, forms of *F. ceranoides* L., *F. vesiculosus* L., or *F. edentatus* De la Pyl. (= '*F. inflatus* L., M. Vahl' of authors), all of which may have irregular inflations in the thallus.

'*Fucus inflatus* L.' is first illustrated by Martin Vahl (1794) in *Flora Danica* (Vol. 7, fasc. 19, tab. 1127); the caption to tab. 1127 is identical with that quoted above from *Syst. Nat.*, and the reference given is 'Lin. S.V., p. 966' (i.e. it is quoted from *Syst. Veg.* of Linnaeus, 1774). However, Vahl adds the following information, presumably from his own knowledge, concerning the distribution of the species: '*Vulgaris in praefectura* Salten, Senjen *et* Tromsöen *Nordlandiae, in aliis partibus Norvegiae non mihi obvius.*'

However, throughout most of the nineteenth century (until Foslie, 1886) the name *F. inflatus* L. was not used for an independent species but, if used at all, was regarded either as a synonym or else as a form of *F. vesiculosus* L.; and, during this period, several new species of *Fucus* were first described, including *F. evanescens* and *F. furcatus* (C. Agardh, 1820), and *F. edentatus*, *F. fueci* and *F. miclonensis* (De la Pylaie, 1829).

Foslie (1886), in proposing that the name *F. inflatus* L. be revived, in particular for those Norwegian plants referred by other authors to *F. edentatus* De la Pyl., recognized that Linnaeus's description 'is no doubt incomplete' ('er vistnok ufuldstaendig'), but suggested that Vahl's illustration and further information on distribution be accepted as delimiting the species in a satisfactory manner, and proposed that the species be cited as '*F. inflatus* (L.) Fl. Dan.'. Foslie adds that certain other early authors (e.g. Lightfoot, 1777) had mistakenly used the name *F. inflatus* L. for certain forms of *F. vesiculosus* L., but suggests that it is very unlikely that Linnaeus, who correctly listed all the other true fucoids of Scandinavia in *Flora Lapponica*, would have set up two species for *F. vesiculosus*.[1] He states that it is highly probable that Linnaeus would have found the species in question at Rørstad; and adds that the epithet *inflatus* doubtless refers to the irregular swellings that are rather common, especially in sterile specimens of this species.

Foslie certainly presents as good a case as possible for retaining the name *inflatus* L. Later European authors, almost without exception, have accepted his views and so we find this species cited as '*Fucus inflatus* L., M. Vahl' by

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[1] Linnaeus, however, does otherwise give two separate specific names to forms of *F. vesiculosus* as at present understood; in *Species Plantarum* (1753) in the section of *Fucus* described as '*dichotomi frondescentes*', we find the following six species: *serratus*, *vesiculosus*, *ceranoides*, *spiralis*, *inflatus* and *divaricatus*. The last-named is merely a form of *F. vesiculosus* (with single vesicles in the axils of all the branches, in the specimens in the Linnaean Herbarium).

Rosenvinge (1893), Börgesen (1902), Jónsson (1903), Kylin (1947) and others up to the present day. However, as the American authors Gardner (1922) and Taylor (1937) have decided that the type specimen of *F. inflatus* L. is a fragment that cannot be associated with any particular species, and use other specific epithets for American fucoids, it is important to resolve this nomenclatural impasse if possible.

For this reason I have been permitted to examine the specimens in the Linnaean Herbarium. The only specimens labelled *F. inflatus* L. in this herbarium are two small pieces of a fucoid mounted side by side on a single sheet (Genus 1274, *Fucus*, Sheet No. 51, in the Catalogue of Savage, 1945), and labelled near the foot of the sheet '5 inflatus' in Linnaeus's handwriting (Pl. I, fig. 1). The pieces of *Fucus* are incomplete (both lack a holdfast) and could well be parts of a single plant; both pieces are completely sterile. The left-hand specimen is 11·7 cm in length with three dichotomies; the lower part of the frond is narrow (3–4 mm) and most of the rest of the frond is less than 10 mm wide, except that midway along the two main branches the frond widens to as much as 17 mm and then becomes narrow again towards the tips. It is suggested that these wider parts are probably the reason for Linnaeus including 'ovato-lanceolata' in his description of the frond. The right-hand specimen is essentially similar, 11·6 cm long, with four dichotomies; and again the rather narrow frond broadens out half-way along its length and then becomes narrower again. The wider parts of both specimens are now pressed flat but some wrinkling of the surface, of the right-hand specimen especially, supports the view that the wider parts were once irregular inflations, such as may be supposed to have occurred in Linnaeus's *F. inflatus* (*cf.* Gardner, 1922, p. 10). It seems probable that Linnaeus, in preparing his later diagnosis in *Syst. Nat.*, had these particular specimens in mind; thus 'punctata' would seem to refer to the rather frequent cryptostomata that are present in both specimens and which appear as prominent raised white spots on the frond surface of the right-hand specimen (Pl. I, fig. 1). Microscopic examination shows that each white spot consists of an aggregation of salt crystals located round the raised orifice of a cryptostoma. Caecostomata (see p. 418) could not be detected even by sectioning a small part of the frond. The fronds are thin and have a very narrow, sharply defined midrib, strongly marked right to the tips of the branches. Near the tips of the left-hand specimen the margins of the thallus appear to be very slightly serrulate; this appearance is caused by marginal cryptostomata, and one of the Linnaean specimens of *F. vesiculosus* (Sheet 48) also shows traces of this character.

These Linnaean specimens of *F. inflatus* closely resemble forms of both *F. vesiculosus* L. and *F. ceranoides* L. as understood to-day and could be interpreted as either of these species. However, having made a critical examination and comparison of the Linnaean specimens of all three species, the author concludes that the specimens labelled '*inflatus*' on Sheet 51 are rather closer to the specimens of *F. vesiculosus* L. than to that of *F. ceranoides* L. and are best interpreted simply as a form of *F. vesiculosus* L. lacking true vesicles.

In the Linnaean Herbarium there are four pages of notes referring to Herb. Linn. specimens, written by Dawson Turner, and it is interesting that he writes of the above specimens: 'one specimen fragment—only a var. of *vesiculosus*' (see also Turner, 1809, pp. 45–7).

The epithets *vesiculosus* L. and *inflatus* L. are both included in *Species Plantarum* (Linnaeus, 1753) and therefore have equal priority but, because *vesiculosus* L. is represented by good specimens in Herb. Linn. (with true vesicles and with unisexual conceptacles), it is desirable that the name *F. vesiculosus* L. be retained for the plant currently known by that name. Turner (1802, 1809) has already clearly united these two taxa under the name *F. vesiculosus* L. and, in accordance with Article 67 of the International Code of Botanical Nomenclature (Lanjouw *et al.* 1952), his choice of epithet must be followed.

Vahl's diagram of '*F. inflatus* L.' in *Flora Danica* (tab. 1127), on the other hand, is certainly of a plant very different from that of Linnaeus, and probably Vahl meant to illustrate the plant widely known now by the later epithet, *edentatus* De la Pyl.[1] The illustration shows a sturdy plant with a broad and prominent midrib running strongly right up to the base of the receptacles; the latter are terminal, rather elongated (4 to 5 times longer than broad), swollen-looking and distinctly pointed. The thallus has a number of irregular inflations, especially in the upper parts. This illustration, despite the much too conspicuous midrib, could be regarded as the earliest publication of the form later widely known as *F. edentatus* De la Pyl.; but the illustration alone could equally well represent a form of *F. vesiculosus* without vesicles and it is therefore recommended that it be disregarded for purposes of typification.

None of the principal defenders and users of the name *F. inflatus* L. have examined the specimens in the Linnaean Herbarium, although, in view of Linnaeus's inadequate diagnosis, the specimens must be decisive. On the other hand, Gardner (1922) obtained a description and sketch of the specimens, and Taylor (1937) examined the specimens personally, and both concluded that these fragments are not sufficiently distinctive to be associated with any particular species. However, neither of these authors mentions that there does exist in the Linnaean Herbarium a sheet of good specimens of the short, very narrow fucoid known as *F. distichus* L. and, since the epithet *inflatus* L. must now be rejected, these specimens assume great importance. If it can be shown that the taxon *F. distichus* L. is taxonomically valid, adequately defined by diagnosis and type specimens, then this name would have priority over all the other names proposed later for the various members of this complex of forms. It will now be shown that the name *F. distichus* L. is valid, but it applies to the very narrow form usually found in littoral pools—the f. '*linearis*' of Rosenvinge, Börgesen and Jónsson—rather than to the short, but more robust, plants developed on very exposed coasts which Börgesen unfortunately and incorrectly named 'f. *disticha* (L.)'.

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[1] See Foslie (1886); also Jónsson (1903) who states in a footnote (p. 184): 'In this connection it may be added that specimens gathered by M. Vahl in Nordland and determined as *F. inflatus* L., fully agreeing with the typical *F. inflatus* L. as it is understood now, are to be found in the Botanical Museum at Copenhagen.'

THE CASE FOR USING THE NAME *FUCUS DISTICHUS* L.

The name *F. distichus*[1] is first used by Linnaeus in *Systema Naturae*, Ed. 12, Tom. 2, p. 716 (1767). The diagnosis given for *F. distichus* is '*Fucus fronde plana dichotoma integerrima lineari fructificationibus tuberculatis mucronatis*'; this may be translated as: '*Fucus* with fronds plane [flat], dichotomously branched, entire [margins], linear [i.e. very narrow], with fructifications [receptacles] having small rounded humps [i.e. conceptacles probably] and sharply pointed.'

This short diagnosis could not by itself delimit any particular species of *Fucus*, and unfortunately Linnaeus does not state where the plant was found. The most significant parts of the diagnosis are the 'linear fronds' and 'pointed receptacles'; 'linear' in the sense used by Linnaeus means very narrow and elongated, and probably also implies uniform breadth.

In the Linnaean Herbarium there are two sheets of *Fucus* specimens labelled 'distichus'; the sheets are numbered '1274.56' and '1274.57', respectively, as stated in the Catalogue of Savage (1945, p. 200).

SHEET 56

Sheet 56 has five pieces of plants pasted on, and is labelled 'distichus' near the foot of the sheet in Linnaeus's hand (Pl. I, fig. 2, pieces labelled A–E by author). These pieces are the true type specimens of *F. distichus* L. and establish the validity of this taxon with certainty. All five specimens are quite short and extremely narrow; in none of them is the holdfast present; the fronds are all compressed and thin, but tough. Specimens A, B and C measure 14·7, 7·8 and 10·8 cm in length, respectively; they are extremely narrow, < 1–2 mm wide, rather cylindrical at the base (< 1 mm), becoming flat above to reach a maximum of 2 mm wide but usually slightly less; A and C have narrow, pointed, terminal receptacles up to 9 mm long and up to 1·5 mm broad, i.e. up to at least 6 times longer than broad; B is sterile.

Specimens D and E measure 7·9 and 8·6 cm in length, respectively, but appear to be only the terminal parts of plants slightly more robust than specimens A–C. The measured width of D and E is not much greater than A–C (fronds *ca.* 2·0 mm wide throughout, but up to 2·5 mm in parts), but the fronds appear to be rather thicker. The receptacles are very well developed, are bigger than in A–C, being up to 20 mm long × 1·5 to 2·0 mm broad, i.e. at least 10 times longer than broad, and are rather pointed at the tips.

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[1] Greek διστιχος, = *distichus*, 'consisting of two rows'; 'distichous' in modern botanical sense, implying branches disposed in two diametrically opposite ranks or rows.

Parts of receptacles from specimens A and E were removed and soaked, and microscopical examination of some fifteen conceptacles showed that all were hermaphrodite; there were 8 eggs per oogonium.

The midrib is rather indistinct in all five specimens, but sections of part of the narrow thallus of A showed that the midrib occupies up to about one-third of the width. Cryptostomata are few and well spaced out in A–C, but rather more frequent in D and E; in D the location of cryptostomata is again indicated by white spots on the frond surface (see p. 411). Caecostomata were quite frequent in A but could be detected only by sectioning the thallus.

Specimens A and C bore frequent calcareous tubes of the worm *Spirorbis borealis* Daudin along most of the length of the specimens; A had a few filaments of a *Ceramium* sp. entangled on the lower part of the lower left-hand branch (see Pl. I, fig. 2); and one small specimen of *Mytilus edulis* L. was found on each of specimens A and D. The delicate habit, and the associated species, of plants A–C in particular, suggest that they may well have been growing in a rock-pool.

The following items are also on Sheet 56: a small label with 'No. 25' written on it (by J. G. Koenig?); and the writing 'linearis Huds. ex syn. Gmel.' and (against the word 'distichus') 'Syst. Nat. [ed.] 12.' in pencil by Sir J. E. Smith.

A comprehensive description of *Fucus distichus* L., accompanied by a very accurate illustration of the Linnaean type specimen (Pl. I, fig. 2 A) is given in Turner (1808, pp. 7–8, and pl. 4), and this is the earliest illustration of the type form of this species that can be accepted without question. Turner describes and illustrates the receptacles and 'tubercles' (= conceptacles) in a detail that is remarkable for his time: 'Fructification situated in the apices of the frond, which are then lengthened to half an inch or more, and become receptacles, containing globular tubercles, placed immediately under their surface, perforated with a small pore, and furnished with a few oblong brown seeds surrounded with a pellucid limbus.' It is clear from his plate 4 (figs. *c*, *d*, and *e*) that the 'oblong brown seeds' are in fact undivided oogonia; his drawings of transverse section conceptacle are not sufficiently detailed to show antheridia, but it should be remembered that antheridia were not properly recognized and described in any species of the genus until much later (Decaisne & Thuret, 1845).

It is practically certain that all five specimens on Sheet 56 are of the same species, and it is quite certain that this is a plant distinct from all known forms of the *Fucus* spp. currently named *F. serratus*, *F. vesiculosus*, *F. spiralis* and *F. ceranoides*; in fact it is *F. distichus* L.

SHEET 57

Sheet 57 of the Linnaean Herbarium is labelled 'Fucus distichus' in Linnaeus's handwriting. The word 'distichus' has been crossed out later and alongside is written 'membranaceus of Stackhouse, D. Turner' in Sir J. E. Smith's handwriting (see Savage, 1945); apparently Smith is quoting Dawson Turner's opinion. Also, in the four pages of notes written by Turner in Herb. Linn., we read: '*F. linearis*,[1] 5 specimens on one paper' (i.e. Sheet 56) 'all the same and right, one on another paper' (Sheet 57) 'a very large specimen of *F. membranaceus*, it was from this latter that Linnaeus was induced to add to his descriptions "Frons nervo medio, textura herbacea".' Following this up, we read as follows in Turner (1808, p. 8, under '*Fucus distichus*')—'It appears by Linnaeus's Herbarium, that he had himself confounded it with *F. membranaceus* of Stackhouse, and this accounts for the observation in *Systema Plantarum* [1779] that the texture is herbaceous.'

'*Fucus membranaceus* of Stackhouse' is the brown alga now known as *Dictyopteris membranacea* (Stackh.) Batt. (=*Haliseris polypodioides* C. Ag.) and it can superficially resemble a plant of *Fucus*. The alga on Sheet 57 is 23 cm long, profusely branched and has thin textured, rather narrow fronds (mostly 2–4 mm broad, but swelling to *ca.* 6 mm when soaked. The author has made a critical examination of this specimen and has found that it is definitely a plant of the genus *Dictyopteris* Lamour., with abundant characteristic male sori; the species could well be *D. membranacea*, although the specimen is much more branched than is usual in this species. The existence of this plant in the Linnaean Herbarium, and the addition to the diagnosis in *Syst. Plant.* (Linnaeus, 1779), could be held to imply that Linnaeus did not have a very clear conception of the delimitation of his *Fucus distichus*. This indeed is very likely and is scarcely surprising. However, this does not alter the fact that his *F. distichus* as originally defined, and particularly as represented by the specimens on Sheet 56 in his herbarium, corresponds precisely to a narrow form of *Fucus* that is widely recognized but which passes under various names today—e.g. *F. inflatus* f. *linearis* (Oed.) Rosenv.; *F. inflatus* f. *linearis* (Huds.) Rosenv.; and *F. filiformis* Gmel. (used by Taylor, 1937). From the taxonomic point of view, it is immaterial that Linnaeus later thought that a plant we know as *Dictyopteris membranacea* should be included in *Fucus distichus* L.; we now place these algae in distinct genera, and the point is covered taxonomically simply by quoting the species as follows: *Fucus distichus* L., *Syst. Nat.*, Tom. 2, p. 716 (1767); syntypes on Sheet 56 in Herb. Linn. (*non* Sheet 57 which plant is certainly *Dictyopteris* and probably *D. membranacea* (Stackh.) Batt.).

Having thus established that the name *Fucus distichus* L. applies to a particular and distinctive fucoid, it remains to confirm that it is either taxonomically distinct from, or else has nomenclatural priority over, several other early names for narrow forms of *Fucus* that have been revived more recently: (i) *F. linearis* Hudson (1762, *Fl. Angl.*, p. 467); (ii) *F. linearis* Oeder (1767, *Fl. Dan.*, tab. 351); and (iii) *F. filiformis* Gmelin (1768, *Hist. Fuc.*, p. 72).

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[1] In these early (but undated) notes Turner was doubtless thinking of *F. linearis* Oeder (*excl. syn.* Hudson) which he later regarded as a synonym of *F. distichus* L. (see also p. 416).

Fucus linearis Hudson (1762, p. 467)

> '*Fucus dichotomus planus linearis vesiculis ovatis sparsis. Anglis*, narrow-leaved Fucus, or Sea-Thongs.' (No illustration).

I fully agree with Turner (1802, p. 128; 1809, p. 45) that *F. linearis* Huds. is a narrow form of *F. vesiculosus* L., with (few or) no vesicles and with elongated receptacles, corresponding to the plant currently known in Britain as *F. vesiculosus* f. *evesiculosus* auctt. In Kew Herbarium there is a sheet of fucoids, originally part of Turner's herbarium, which includes two small pieces of a fucoid labelled '*Fucus linearis* Huds.—from himself—H.D. I divide with you an indifferent specimen'. This is material given by Hudson himself to the Rev. H. Davies who, to quote Turner (1802, p. 128), 'was kind enough to divide with me [Turner] an original specimen'. The present author has made a critical examination of this most interesting specimen and confirms that it is indeed a (male) plant of *F. vesiculosus* 'f. *evesiculosus*', without vesicles. In view of this finding, it is clear that the name 'f. *evesiculosus*' is nomenclaturally superfluous, and the narrow, reduced form of *F. vesiculosus*, with few or no vesicles, found on exposed and semi-exposed coasts, should properly be named *F. vesiculosus* L., f. *linearis* (Huds.).[1]

Fucus linearis Oeder (1767)

Tab. 351 in *Flora Danica* (Vol. 2, fasc. 6, 1767, edited by G. C. Oeder) shows a rather idealized drawing of a fucoid certainly very similar to *F. distichus* L. The caption referring to the plate (on p. 9) states:

> '*Fucus marinus secundus*. Dod. Pemt. 479. *Fucus*, linearis, *dichotomus planus linearis acutus, vesiculis ovatis sparsis*. Huds. Angl. 467. Locus. *In fundo oceani Islandici*.'

Oeder is thus using Hudson's name *linearis* and quotes Hudson's diagnosis, but gives Iceland as the locality. The name *linearis* Huds. has just been shown to refer to a plant taxonomically quite distinct from *F. distichus* L., but if Oeder's sketch is indeed of a plant from Iceland then it could well be of true *F. distichus* L. I am inclined to agree with Turner (1808), Lyngbye (1819) and Hornemann (1827) that the sketch (only) of *F. linearis* in *Flora Danica* can be accepted as an illustration of *F. distichus* L. and may be cited in this respect in the following manner (='*F. linearis*' *Flor. Dan.*, tab. 351, *excl. syn.* Hudson).

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[1] Batters (1902, p. 50) is therefore in error in supposing *F. linearis* Huds. to be the curious, very narrow form of *F. ceranoides* L. apparently recorded in Britain only from Loch of Stenness, Orkney Islands, where it grows in rather unusual environmental conditions (for recent information on the flora and physical conditions in Loch Stenness, Orkney, see Dunn, 1937, and Nichol, 1938). I have seen authentic specimens of this form of *F. ceranoides* from Loch Stenness in the Greville Herbarium (University of Edinburgh) and there is no doubt that it really is a form of *F. ceranoides*, with extremely thin, narrow fronds, and with the midrib very distinct and fine; the receptacles are elongated (but not markedly so) and the plants are dioecious. Lightfoot's (1777, p. 912) record of '*F. distichus* L.' from 'Loch Stennis, Orkney' also almost certainly refers to this same narrow form of *F. ceranoides*.

Fucus filiformis Gmelin (1768)

This name has been revived by Taylor (1937) for a narrow fucoid found in littoral pools in North-east America. However, again we find that Gmelin (p. 72) is merely quoting a name used for the first time by Hudson (1762, p. 472), who gives the following diagnosis for this plant: '*F. filiformis dichotomus planus. Anglis*, flat Fucus. *Habitat in littore Lancastriensi*' [Lancashire coast]. In the second edition of *Flora Anglica*, however, Hudson (1778, p. 585) gives an expanded diagnosis and additional information about '*F. filiformis*', as follows: '*Fucus fronde cartilaginea filiformis compressa dichotoma acuta. Anglis*, filiform Fucus. *Habitat in rupibus et saxis prope insula* Walney *in comitatu Lancastriensi.* Desc. *Frons semipedalis, cartilaginea, filiformis, compressa, dichotoma, diaphana, rubescens.*' This almost certainly refers to a red alga, and probably to a form of *Chondrus crispus* (L.) Stackh. Gmelin (1768) repeats Hudson's (1762) earlier diagnosis verbatim and adds some further description; for location he states '*Oceanus septentrionalis*' [northern Ocean] and he also gives a drawing of his conception of this plant (1768, tab. 1 A, fig. 1). Other authors (e.g. Turner, 1808, though with some doubts) have quoted this illustration (but without the diagnosis and reference to Hudson) as a further possible illustration of *Fucus distichus* L. However, I think that the drawing is not accurate enough to be cited in this connexion; in particular, Gmelin's figure shows the midrib altogether too narrow and well defined, and clearly distinct all the way to the tips of all the branches (including running along the length of what are presumably rather flat-looking receptacles, a phenomenon that I have otherwise seen only in a few young receptacular apices of British *F. ceranoides*).

Thus the name *F. filiformis* (Hudson, 1762, 1778, Gmelin, 1768) has been used with different meanings and cannot now be associated with any particular type; it should therefore be rejected (Lanjouw *et al.*, 1952, Article 73). On the other hand, Taylor's (1937) own description and illustration of North-eastern American plants correspond precisely with the plants of *F. distichus* L. in Herb. Linn. and I have no doubt that they should be so named. (The nomenclature of North American fucoids will be discussed further on a later occasion.)

FUCUS DISTICHUS L. EMEND. POWELL

It has been shown above that the name *F. distichus* L. applies to a distinctive narrow fucoid that can be regarded as a good species. It is now proposed that the specific limits of *F. distichus* L. be amended to include the various hermaphrodite fucoids reduced to synonymy below. In the present treatment four of the many described forms are considered as subspecies. Each subspecies is fairly distinct from the others, at least in some parts of the species range. Near the centres of distribution, however, all possible intermediate forms between the four main subspecies may be found.

GENERAL CHARACTERS OF THE SPECIES AS A WHOLE

The species as a whole is extremely plastic and, in response to varying environmental conditions and in different parts of its very wide geographical range, develops into a great variety of forms, the over-all range of form being indicated in the descriptions of subspecies below. Two particular characters, however, are fundamental to the present conception of the species: (i) the conceptacles of all forms are invariably hermaphrodite; (ii) closed cavities, termed 'caecostomata', are found in variable number in the fronds of most forms of the species, but not in any other species of the genus *Fucus*. The author cannot yet state that caecostomata are invariably present in all forms, but they have been detected (sometimes only in very small numbers) in all specimens so far examined for this character. In view of their taxonomic importance in a group (*Fucus* spp.) in which diagnostic characters are few, caecostomata are dealt with in detail.

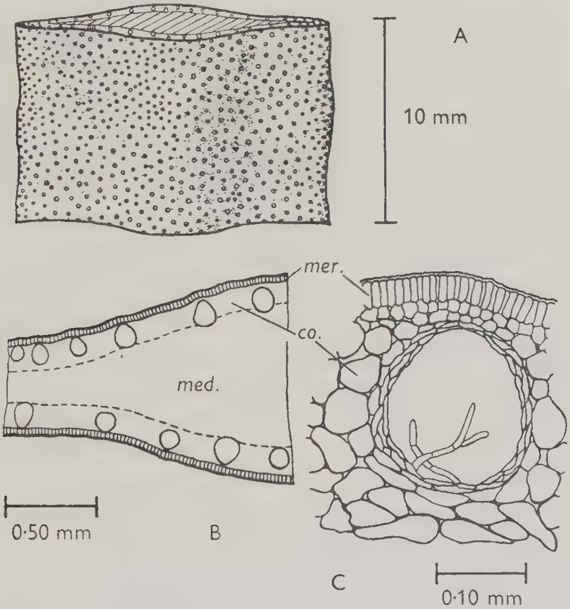
Caecostomata

The descriptive term caecostomata was apparently first suggested by Prof. W. A. Setchell, and first used in print by Gardner (1922), for small completely closed cavities found in the fronds of certain Pacific coast fucoids referred by Gardner to the species '*F. furcatus* Ag.' (and '*F. edentatus* De la Pyl.'). Caecostomata are mentioned otherwise only by Setchell & Gardner (1925), Smith (1944), Fritsch (1945, p. 368, quoting Gardner, 1922), and Doty (1947)—all referring to Pacific coast material. However, Gardner (*loc. cit.*, p. 9) suspects that J. Agardh (1868, p. 38) may have been referring to them under '*Cryptostomata immersa saepe numerosa, plurima diu clausa*' [immersed (sunken) cryptostomata often numerous, most long since enclosed] with reference to his (J. Agardh's) conception of *F. filiformis* Gmelin, *F. linearis Flor. Dan.* and *F. miclonensis* De la Pyl.; this may be so, and J. Agardh repeats the above description (p. 40) with reference to the following fucoids: *F. edentatus* De la Pyl., *F. furcatus* C. Ag. and *F. evanescens* C. Ag. (De Toni, 1895, repeats the J. Agardh description for these same fucoids). Gardner also states (p. 18), very significantly, that he found 'a large number of caecostomata' in some of Börgesen's specimens of '*F. inflatus* f. *edentata*' from the Faeröes.

I have found caecostomata in all of the North Atlantic forms of *F. distichus* L. emend. Powell, and disagree with the taxonomic conception of Gardner (1922) that the presence of caecostomata can be used as a 'fundamental distinguishing character' of his *F. furcatus* Ag. emend.

In British material of *F. distichus* subsp. *edentatus* the presence of caecostomata may readily be detected (in both fresh and herbarium material) simply by holding a frond up to a light, when the internal cavities appear as small, lighter coloured (less dense), rounded spots in the frond, and, when the caecostomata are very numerous, the frond appears to be stippled with small light-coloured dots. Also, the meristoderm and cortex form a slight hump above each cavity, so that the frond surface appears to be covered with low, rounded, miniature pimples and has the appearance of a very fine-grained morocco-leather. The caecostomata (Text-fig. 1) are, in general, both more numerous and smaller near the thallus margins. The cavities are lined with flattened cells, are located at first mostly in the cortex (later extending a little deeper, into the outer medulla), and vary a good deal both in shape and size: the shape is most often pyriform, or rounded, or sometimes a rather flattened pear-shape; the size of the cavities is usually (50–) 100–200 (–250) μ greatest diameter. Gardner (1922) states that 'no paraphyses develop' in the caecostomata he examined, but investigation of British material has shown that reduced, colourless paraphyses may develop to a small extent in some few of the caecostomata (Text-fig. 1 C), although the paraphyses are sometimes more numerous than shown in the figure. The paraphyses may be slightly branched.

The development of caecostomata has been investigated, by sectioning and squashing thallus apices, and my observations confirm those of Gardner that caecostomata originate in the same way as do the conceptacles and ordinary cryptostomata in *Fucus* spp. (see Fritsch, 1945, for a review of the literature relating to the origin and development of these structures; Fritsch refers to cryptostomata as 'cryptoblasts'). All three structures originate just behind the growing point from a single initial cell. This soon becomes lodged at the base of a deep and narrow cavity owing to rapid growth and division of the surrounding cells. The cavity then enlarges and becomes lined with several layers of flattened cells, which delimit these structures from the compact cortical tissue and loose medullary tissue.



Text-fig. 1. Caecostomata in *Fucus distichus* subsp. *edentatus* (plant from Lerwick harbour, Shetland Islands). (A) Surface view of part of thallus; the circles represent (internal) caecostomata. (B) Transverse section of part of thallus, showing caecostomata situated mostly in the cortex. (C) Enlarged diagram of a single caecostoma, containing two colourless paraphyses. *co.*, cortex; *med.*, medulla; *mer.*, meristoderm.

From this point onwards, the development of caecostomata differs from that of cryptostomata and conceptacles. The pores communicating with the exterior become blocked by cell-division of the meristoderm very close behind the apex (within the first few mm), and the cavities soon become completely roofed over. In the older parts of the thallus the cavities are seen to have become very much larger, and to be roofed over by slight mounds of tissue (consisting of flattened limiting cells, a few layers of cortical cells, and meristoderm), and appearing as small mounds on the surface of the frond.

It would seem best to interpret the caecostomata as small, reduced, closed cryptostomata, although cryptostomata never occur (in any species of *Fucus*) at anything like the density that caecostomata may achieve.

In British *F. distichus* subsp. *edentatus*, caecostomata are often extremely abundant (up to a maximum density of 500 per cm^2), and cryptostomata few or absent; while in the Linnaean type of *F. distichus* subsp. *distichus*, and generally in *F. distichus* subsp. *anceps* (both with very reduced fronds), both caecostomata and cryptostomata occur only in small numbers and the caecostomata often can be detected only by sectioning the frond.

DESCRIPTION AND GENERAL DISTRIBUTION OF SUBSPECIES

Subsp. *distichus*. (Pl. I, fig. 2)

Fucus distichus L., *Syst. Nat.*, Ed. 12, Tom. 2, p. 716 (1767), Turner (1808, pp. 7–8, but excl. some synonymy); probably *F. distichus* f. *b tenuior* J. Agardh (1868). [*Non F. distichus*, Lightfoot, 1777 (= form of *F. ceranoides* L., see footnote on p. 416), Carruthers, 1863 (= *F. distichus* subsp. *anceps*).]

F. linearis, Oeder (1767, tab. 351). (*Non F. linearis* Hudson, 1762, see p. 416.)

F. filiformis, De la Pylaie (1829), Farlow (1881), Taylor (1937), A. D. Zinova (1953), E. S. Zinova (1954*a*, *b*); but may not be the type of *F. filiformis* Gmelin (1768) (see p. 417).

F. inflatus var. *linearis* (Oed.) Rosenvinge (1893); *F. inflatus* f. *linearis* (Oed.) Börgesen (1902), Norum (1913), Printz (1926), Powell (1954); *F. inflatus* f. *linearis* (Huds.) Jónsson (1903). (*Non F. inflatus* f. *disticha* (L.) Börgesen, 1902.)

Nomenclatural type. Syntypes are on Sheet 56 in Herb. Linn. (*non* Sheet 57); Sheet 56 is here reproduced as Pl. I, fig. 2. To avoid any possible ambiguity in future, the uppermost specimen labelled A in the photograph (Pl. I, fig. 2A) is now designated the lectotype. It is not known where the syntypes were collected.

Illustrations. The lectotype is very accurately illustrated by Turner (1808, pl. 4). Oeder's illustration (1767, tab. 351) is not so accurate. Taylor (1937, pl. 23, fig. 2); and A. D. Zinova (1953, p. 184, fig. 142).

Description. Plants relatively small and delicate, 5–15 (–20) cm in length (up to 40 cm according to A. D. Zinova, 1953), light brown; holdfast relatively small; stipe very thin, lax, round or oval in section; branching dichotomous and usually distichous, the axils rather acute; branches with entire margins, evesiculate, narrow, linear, 1·5–3 (–4) mm wide, thin; principal branches with a definite but not very prominent midrib and narrow lateral alae; in the lower parts of the plant the midrib becomes a little narrower but somewhat thicker, forming the thin stipe, while towards the tips of the branches the midrib usually becomes indistinct; cryptostomata and caecostomata both usually present, but few, small and obscure. Receptacles apical, narrowly cylindrical to fusiform, generally inflated, broader than the distal parts of the fronds which bear them, 0·5–3 cm long and 1–4 mm broad, unbranched or once-forked; conceptacles hermaphrodite.

Distribution. Kara Sea (A. D. Zinova, 1953); Barents Sea; White Sea; N. and W. Norway; Iceland; Faeröe Islands; Greenland; Atlantic coast of Canada and U.S.A.; Sea of Okhotsk and Gulf of Tartary (E. S. Zinova, 1954*a*, *b*). Not recorded (and probably absent) from British Isles. This plant largely occurs in rock-pools (but may occur also on open rock in some northern parts of its range) in the upper part of the littoral zone, at both exposed and sheltered sites.

Authentic specimens examined.[1] The syntypes in Herb. Linn. (LINN) have been examined in detail; it is not known when, where, or by whom, the specimens were collected. **N.W. Russia**. 'Terra parva Samojedorum: Cap Barmin, August, *Dr* [F.] *Ruprecht*' [as '*F. vesiculosus* L. (f. *nana*)'] (K, ex Herb. Hooker). WHITE SEA: *F. Ruprecht* (K, same sheet). (Also on this sheet in Herb. Kew are some authentic specimens of subsp. *distichus* labelled '*Fucus distichus* ex herb. Linn.; Mr. Stackhouse from M. Der Fontaine 1802'.) **Norway**. FINMARK: Vardöhus (TCD); (also in Herb. Harvey, TCD, is another sheet of authentic material labelled '*F. distichus* L., ex Herb. Agardh'—presumably these are the 'authentic specimens' sent to W. H. Harvey by J. Agardh—see Carruthers, 1864); Gjesvaer, 1880, *M. Foslie* (BM, ex Herb. Holmes); 'Norv. arct., Mehavn,' May 1882, *F. R. Kjellman* (BM, ex Herb. Batters); 'West Finmarken, Maaróe' (?), July 1867, *Th. M. Fries* (BM, ex Herb. Batters). LOFOTEN: Reine, May 1952, *E. Baardseth* (MILL). NORDMÖRE: Bud, July 1955, in pools, *E. Conway* (GL). **Iceland**. Sept. 1897, *H. Jónsson* (K, *Plantae islandicae*). **Faeröe Islands**. SYDERÖ: Famien, May 1896, *F. Börgesen* (BM, *Algae marinae Faeroenses*, Nr 501 a, two sheets—one ex Herb. Batters, the other ex Herb. Holmes). **Canada**. NOVA SCOTIA: Peggy Cove (near Halifax), Aug.–Sept. 1948, *T. A. & A. Stephenson* (Herb. M.S. Doty, No. NSP. 14, two sheets).

Subsp. *anceps* (Harv. et Ward ex Carruthers) Powell, *n.comb.*
(Pl. II, fig. 1)

Fucus anceps Harv. & Ward, Carruthers (1864), Gray (1867), Batters (1902), Newton (1931); *F. anceps* Ward & Harv., Harvey (1864); *F. anceps* 'Wood & Harv.', J. Agardh (1868); *F. anceps* 'Harv. et Wood', Areschoug (1868).

F. distichus, Carruthers (1863, excl. synonymy and distribution), Du Rietz (1947), A. D. Zinova (1953); probably *F. distichus* f. *a robustior* J. Agardh (1868).

F. inflatus, Arwidsson (1937), Parke (1953); *F. inflatus* f. *disticha* (L.) Börgesen (1902, but excluding some of his synonyms), Norum (1913), Printz (1926), Hygen & Jorde (1935), Levring (1937); *F. inflatus* f. *distichus* (L.) Börg., Powell & Lewis (1952), Gauld *et al.* (1953), Burrows *et al.* (1954), Lewis (1954); *F. inflatus* f. *exposita* Jónsson (1903), Powell (1954).

The name *Fucus anceps* Harv. & Ward ex Carruthers refers to a small form of *Fucus* found growing on the very exposed face of Duggerna Rock, Kilkee, West Ireland, by Prof. W. H. Harvey and Mr N. B. Ward on 19 July 1863. The plant was first briefly reported as '*F. furcatus* Ag.' (Anon., 1863), then as '*F. distichus* L.' (Carruthers, 1863, who described and illustrated the plant in detail). However, after examining authentic specimens of *F. distichus* L. sent to him by J. Agardh, Harvey finally decided that the Irish plant was 'a distinct and hitherto undescribed species' for which (in a letter to Carruthers) he proposed the name *F. anceps* Harv. & Ward (see Carruthers, 1864)[1]; the Latin word *anceps*, means 'two-headed', or 'two-fold' and was considered appropriate by Harvey (1864) because 'this Fucus seems to combine the characters of the ribbed and ribless species'. Additional details relating to the original discovery of *F. anceps* at Kilkee are given in notes and correspondence published in *Trans. bot. Soc. Edinb.*, Vol. 8, 1866 (pp. 52, 53, 111). I propose to revive the name *anceps* for this plant of very exposed coasts because the good description and figures of *F. anceps* quoted above are the earliest really certain account of this very distinctive subspecies.

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[1] The abbreviations used for herbaria are as follows: (BM) British Museum; (E) Royal Botanic Garden, Edinburgh; (GL) University of Glasgow; (K) Royal Botanic Gardens, Kew; (LINN) Linnaean Society of London; (MILL) Scottish Marine Biological Association, Millport; (TCD) Trinity College, Dublin.

Nomenclatural type. The description of this plant given in Carruthers (1863) is based on a series of syntypes from Kilkee, now lodged in the Herbarium of the British Museum. One specimen is closely similar to the principal illustration in Carruthers (1863, tab. 12, fig. 1) and I select this specimen as the lectotype, even though it is sterile.

Illustrations. Carruthers (1863, tab. 12, figs. 1–9); Börgesen (1902, p. 471, fig. 93, this illustration is reproduced by A. D. Zinova, 1953, p. 191, fig. 146); Printz (1926, p. 210, fig. 25); present work, Pl. II, fig. 1.

Description. Plants relatively small but very sturdy, usually (4–) 6–10 (–15) cm in length; yellowish brown to dark brown; holdfast well developed, up to 2 cm diam., giving very firm attachment; stipe short, relatively very thick, almost terete and stands erect, while the more lax distal branches arch over very characteristically; branching distichous, and usually dichotomous but may be unilateral in part; the angle between older branches is often rather wide and between youngest branches very acute, so that the young terminal branches are closely crowded and run almost parallel; branches with entire margins, evesiculate, alate above but narrow, seldom more and usually less than 4 mm wide, thicker than in subsp. *distichus* and consisting mainly of a stout midrib, with very narrow lateral alae on each side; towards the tips the branches become narrower, the midrib and alae are less distinct and the frond becomes more nearly oval in section; on older branches the alae are usually absent; a small number of cryptostomata present, often as a single row on each side of midrib in the younger thalli; in older branches, the hairs of the cryptostomata are often worn down to the level of ostiole; caecostomata present in small numbers in all specimens examined, but often their presence can only be detected by sectioning. Receptacles apical, elongated, narrowly cylindrical and often slightly curved (continuation of arching of frond), generally inflated, always broader than the distal parts of the frond which bear them, usually 1·5–3 cm long and 2–3 mm wide, but occasionally up to 4 cm long and 4 mm broad, unbranched or once-forked, apices bluntly pointed and often sterile, and continued vegetative growth beyond the receptacle is often seen; conceptacles invariably hermaphrodite. Often all the apices of a frond are fertile at the same time. Occasionally irregular areas of fertile tissue (often occupying only half the width of the frond, or less) may be found some way back from the apex; this character was first noticed by Carruthers (1863, p. 354, and tab. 12, fig. 7) and described as an 'inferior lateral receptacle' (*cf.* description of subsp. *edentatus* below).

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[1] In another letter reporting the new name, Harvey (1864) cites the authorities in reverse order, i.e. as 'Ward & Harvey'. I propose to retain the order of authorities given in Carruthers (1864).

Distribution. North-west Russia (Barents Sea and White Sea) according to A. D. Zinova (1953, p. 191) who describes the plant under the name *F. distichus*; North, West and South Norway; Iceland; Faeröe Islands; British Isles (North and West Scotland and Ireland only). This plant is found only on very exposed coasts, subjected to considerable swell and wave-action, in the upper part of the littoral zone.

Authentic specimens examined. **Norway.** NORDMÖRE: Bud, July 1955, *E. Conway* (GL), *G. G. Smith* (MILL). **Faeröe Islands** (all specimens collected and distributed by F. Börgesen). MYGGENAES HOLM: July 1902 (BM, *Algae marinae Faeroenses*, ex Herb. Holmes; K, *Alg. mar. Faer.*, ex Herb. Börgesen). STORE DIMON HOLM: June 1896 [BM, *Alg. mar. Faer.*, two sheets—one (No. 895) ex Herb. Batters, the other ex Herb. Holmes]. SYDERÖ: Vaags Ejde, June (K, *Kryptogamae Exsicc.*, No. 1746); Sumbö Holm, July 1899 (BM, *Alg. mar. Faer.*, No. 1617*a*, ex Herb. Holmes; K, *Alg. mar. Faer.*). **Scotland.** SHETLAND ISLANDS: Fair Isle: (i) North Gavel, (ii) reefs S.W. of South Lighthouse, June 1952, *H. T. Powell* (MILL, GL). ORKNEY ISLANDS: Sandwick, Mainland: (i) Hole o'Rowe, Oct. 1938, and (ii) near Garson, May 1939, *J. Sinclair* (Herb. J. Sinclair, Sheets No. 445 and 511, respectively). CAITHNESS: North coast: (i) near Crosskirk, (ii) Lower Dounreay, (iii) near Sandside Head, etc., July 1951, *H. T. Powell* (MILL, BM, K). OUTER HEBRIDES: Island of Lewis: (i) 'Butt of Lewis' [?], April 1909, *W. J. Gibson* (K), (ii) Buaile na Faing (half-mile N. of Port of Ness), July 1954, *H. T. Powell* (MILL); St Kilda Islands: Glen Bay, Hirta, July 1952 and July 1956, *T. B. Bagenal* (MILL). **Ireland.** DONEGAL: Malin Head, July 1953, *J. R. Lewis* (MILL). CLARE: Duggerna Rock, Kilkee, (i) July 1863, *W. H. Harvey & N. B. Ward* [BM (syntypes and lectotype), E, K, TCD], (ii) July 1953, *H. T. Powell* (MILL); Kilkee, (i) *J. Cook* (BM, ex Herb. Holmes), (ii) Sept. 1897, *E. George* [BM, K, etc., a large collection of sterile plants widely circulated as Holmes's *Algae Britannicae Rariores Exsiccatae*, Fasc. X, No. 240]; also from (i) Donegal Point, (ii) George's Head, (iii) reefs just N. of Goleen Bay, and (iv) four sites just N. of Ross Bay, July 1953, *H. T. Powell* (MILL). KERRY: Kerry Head, June 1953, *J. R. Lewis* (MILL).

Subsp. *edentatus* (De la Pyl.) Powell, *n.comb.*
(Plate II, fig. 2)

Fucus edentatus De la Pylaie (1829, p. 84, *excl. syn.*), J. Agardh (1868), Gardner (1922), Setchell & Gardner (1925), Taylor (1937), Lund (1949*a*, *b*), A. D. Zinova (1953, 1954); *F. edentatus* f. *typica* Kjellman (1883).

F. inflatus, probably Vahl (1794, tab. 1127), Foslie (1886), Kjellman (1890), Printz (1926), Kylin (1947), Sundene (1953); *F. inflatus* f. *typica* Jónsson (1903); *F. inflatus* var. *a edentatus* (De la Pyl.) Rosenvinge (1893); *F. inflatus* f. *edentata* (De la Pyl.) Börgesen (1902, 1903); *F. inflatus* f. *edentatus*, Burrows *et al.* (1954). (*Non F. inflatus* L., see pp. 409–12.)

F. furcatus C. Agardh (1820, 1821), J. Agardh (1868), Kleen (1874), Farlow (1881), Gardner (1922, in part), Setchell & Gardner (1925, in part); (= *F. Gardneri* Silva, 1953)[1].

F. evanescens, Gardner (1922, in part), Setchell & Gardner (1925, in part).

F. nitens Gardner (1922), Setchell & Gardner (1925).

Nomenclatural type. Not seen by the present author. According to De la Pylaie (1829, p. 84) type material from Newfoundland (De la Pylaie, Herb. Terre-Neuve) was deposited in the Herbarium of the Paris Museum. Gardner (1922, p. 11) confirmed this and published a photograph (his plate 60, fig. 1) of the 'type specimen', which is presumably the holotype.

Illustrations. Kützing (1860, tab. 17 II); Börgesen (1902, pp. 467–9, figs. 90–92; 1905, p. 747, fig. 158), (1904, p. 56, fig. 7); Gardner (1922, pl. 60, fig. 1, also pls. 4, 10, 13, 18–23, 25, 54); Hylmö (1933, p. 383, fig. 4, uppermost plant); Taylor (1937, pl. 23, fig. 3); Levring (1946, fig. on p. 192); Kylin (1947, taf. 16, fig. 2); Lund (1949*a*, p. 233, fig. 1); A. D. Zinova (1953, p. 186, fig. 144); present paper, Pl. II, fig. 2. (The illustrations referred to by De la Pylaie, 1829, were unfortunately never published.)

Description. Plants typically large and sturdy, 20–45 (–60; and exceptionally –90, Taylor, 1937) cm in length; dark brown; branching regularly dichotomous and usually distichous, axils generally acute; branches with entire margins, evesiculate, leathery and more or less flaccid, alate above, but rather narrow for a fucoid of this size, (5–) 9–15 (–20) mm broad but a little broader just below the dichotomies, relatively quite thick, midrib very distinct below but may become very indistinct above (especially immediately below the receptacles); irregular inflations may occur in the lateral alae especially in distal segments; towards the base the midrib is denuded of alae and forms a firm stipe; holdfast a broad conical disk; cryptostomata typically few but can be quite numerous, usually rather small and inconspicuous in older branches; caecostomata usually present, from few to very abundant (up to 500 per cm^2). Receptacles apical, typically elongated and swollen, cylindrical or somewhat flattened, 2–10 (–22, Gardner, 1922, p. 50) cm long, 5–15 (–25, Gardner, 1922) mm broad below, often tapering to acute (sterile) tips, usually divided 1–3 times into antler-like subdivisions, often not very sharply demarcated from sterile tissue below; vegetative growth beyond the receptacle may often occur (see Pl. II, fig. 2); conceptacles invariably hermaphrodite. Occasionally irregular areas of fertile tissue (often occupying only half the width of the frond, or less) may be found some way back from the apex (*cf.* subsp. *anceps*). When dried, the plants become very dark brown or black in colour and the receptacles of herbarium specimens are usually pressed quite flat; the midrib may appear more prominent in dried specimens than in living plants.

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[1] Silva (1953) proposed the new name *F. Gardneri* for *F. furcatus* C. Ag., because the latter name is a homonym of *F. furcatus* Esper [1800, p. 178 (138 by error), plate 95] and is therefore nomenclaturally illegitimate; according to Silva, 'Esper's *Fucus furcatus* was based on a plant from the Adriatic Sea...most likely referable to the alga currently known as *Fauchea repens* (C. Ag.) Mont.'.

Distribution. North-west Russia (Barents Sea and White Sea) according to A. D. Zinova (1953, p. 185); North and West Norway, Oslofjord, West Sweden, Copenhagen; Iceland; Faeröe Islands; British Isles (only in Shetland Islands and Fair Isle); Greenland; Atlantic coast of Canada and U.S.A.; Pacific coast of Canada and U.S.A.; Kamchatka; North Japan. This plant is typically a good deal larger than the two preceding subspecies, and it grows best at sheltered or semi-exposed sites, in the sublittoral fringe and lower midlittoral zones. It may be found, however, at any level from the upper midlittoral zone down into the sublittoral zone; the plants growing at high levels or in more exposed situations are generally shorter and narrower.

Authentic specimens examined. **Norway.** TROMS: Tromsö, June 1887, *M. Foslie* (K, *Hauck et Richter Phykotheka universalis*, No. 164). LOFOTEN: Reine, May 1952, *E. Baardseth* (MILL). S. TRÖNDELAG: Trondheimsfjord, July 1955, *H. Blackler* (GL). AKERSHUS: Oslofjord, May 1953, *O. Sundene* (MILL). **Iceland.** 1897, *H. Jónsson* (K, *Plantae islandicae*). Skalaness, Aug. 1952 (GL). **Denmark.** Copenhagen harbour, May 1951, *S. Lund* (MILL). **Faeröe Islands.** STRÖMÖ: (i) Thorshavn, June 1895 (No. 224), (ii) near Kalbakfjord, June 1898, *F. Börgesen* (BM, *Algae marinae Faeroenses*, ex Herb. Batters). **Scotland.** SHETLAND ISLANDS: Mainland: Lerwick, (i) '16. 6. 1902', *F. Börgesen* (K), (ii) June 1908, *W. A. Russell* (BM, K, Holmes's *Algae Britannicae Rariores Exsiccatae*, Fasc. XII, No. 288), (iii) June–July 1952, *H. T. Powell* (MILL, GL); Scalloway harbour, July 1952, *H. T. Powell* (MILL). Fair Isle: North Haven, June–July 1952, *H. T. Powell* (MILL, GL). **Canada.** NOVA SCOTIA (all specimens collected Aug.–Sept. 1948, by *T. A. & A. Stephenson*, and now in Herb. M. S. Doty): (i) Halifax harbour (Herb. No. NSH. 13, one of three sheets labelled *F. evanescens*); (ii) Peggy Cove (Herb. No. NSP. 14, two sheets); (iii) Hall's Harbour, Minas Channel, Bay of Fundy (Herb. No. NSBF. 5, two sheets). BRITISH COLUMBIA: Vancouver Island: Beacon Hill, Victoria, June 1908, *J. Macoun* (BM, ex Herb. Geological Survey of Canada). **U.S.A.** MAINE: Eagle Island, Penobscot Bay, July 1896, *F. S. Collins* (BM, *Phycotheca Boreali-Americana*, Collins, Holden & Setchell, XIII). MASSACHUSETTS: Nahant (BM, ex Herb. F. S. Collins).

Subsp. *evanescens* (C. Ag.) Powell, *n.comb.*

Fucus evanescens C. Agardh (1820, 1821), J. Agardh (1868), Kjellman (1883), Yendo (1907), Gardner (1922, in part), Setchell & Gardner (1925, in part), Taylor (1937), E. S. Zinova (1933, 1954*a*, *b*, *c*), A. D. Zinova (1953).

F. inflatus var. *evanescens* (C. Ag.) Rosenvinge (1893); *F. inflatus* f. *evanescens* (C. Ag.) Jónsson (1903).

Nomenclatural type. Gardner (1922, p. 12) states: 'according to Setchell, the type specimen is in the herbarium of J. G. Agardh, under No. 00299, with a query.' I have not seen this (apparently doubtful) specimen and, for the present, temporarily designate as the type C. Agardh's description (1820, pp. 92–3) and illustration (1821, plate 13). C. Agardh's illustration is very well reproduced, photographically, by Gardner (1922, plate 1, fig. 2). For location, Agardh (1820) writes: 'Ad Sachalien, Tilesius; ad Kamtschatka, Chamisso; unde specimina communicaverunt' (whence specimens have been communicated); and the locality of the specimen illustrated by Agardh (1821, plate 13) is also stated to be Kamtschatka. E. S. Zinova (1933, 1954*c*) also records this alga from Kamchatka.

Illustrations. C. Agardh (1821, plate 13); Gardner (1922, plate 1, fig. 2; also plates 11, 11a, 35, 36, 45, 46, 47, 48, 52, 56, 58, 59); Taylor (1937, plate 23, fig. 4; plate 24, fig. 2); A. D. Zinova (1953, fig. 147).

Description. This subspecies is the most variable and therefore the most difficult to define of the four subspecies recognized in the present paper. The principal features of *F. evanescens*, as originally defined and drawn by C. Agardh (1820, 1821), were: plant large, fronds broad; receptacles flattened, not markedly elongated, relatively broad; midrib indistinct in the apical parts of the plant. Some authors (e.g. Rosenvinge, 1893; Jónsson, 1903; Taylor, 1937) have applied the name to large, relatively broad, plants usually found in the lower part of the littoral zone (and in sheltered situations) in some of the more southerly parts of the over-all range of the subspecies; these large plants typically have fronds wider and receptacles shorter than subsp. *edentatus*, and correspond most nearly to the type illustration. Other authors (e.g. Kjellman, 1883; Gardner, 1922; A. D. Zinova, 1953) have applied the name to a number of relatively small and often rather narrow plants found on Arctic and other northern shores. As in subsp. *edentatus*, characters such as the midrib becoming indistinct above, and the numbers of cryptostomata and caecostomata, are in fact very variable; and, if we include the narrow Arctic forms, even the relative width of the frond varies widely. Indeed, the only characteristic morphological features of subsp. *evanescens*, throughout its geographical range, relate to the shape of the receptacles—relatively short and broad, rather flattened, and fairly distinctly delimited from the rest of the frond (*cf.* subsp. *edentatus*). From several Arctic areas, only plants with such receptacles have been reported (it is apparently the only subspecies that is circumpolar in distribution) and such plants also are not found so far south as, for example, subsp. *edentatus*; it is therefore convenient to recognize subsp. *evanescens* as the subspecies best adapted to Arctic conditions, even though the morphological distinction from forms of subsp. *edentatus* is somewhat arbitrary, and despite the fact that the small distinctions between these two subspecies are obscured in some parts of the world by the presence of all possible intermediate forms, with receptacles intermediate in shape sometimes even on the same plant.

According to Inoh (1935), material of '*F. evanescens* Ag.' collected near Muroran, Hokkaido, Japan, has a haploid chromosome number of 32; this is the number usually reported for *Fucus* spp.

Distribution. Arctic and subarctic, circumpolar. Siberian Polar Sea, N.W. Russia, Novaya Zemlya, Svalbard, Jan Mayen, Bear Island; Greenland, Iceland; American Polar Sea, Atlantic Canada, Atlantic U.S.A.; Pacific Canada, Pacific U.S.A., Bering Sea, Aleutian Islands, Kamchatka, Sea of Okhotsk, Kurile islands, North Japan. *F. distichus* subsp. *evanescens* has not been found in Britain; the nearest locality is probably Iceland (Jónsson, 1903), although Börgesen (1902, p. 471) mentions finding a few plants possibly referable to subsp. *evanescens* at Thorshavn (Faeröes). The larger forms of this subspecies are found near or below low tide level at sheltered or semi-exposed sites. The smaller forms are found under more rigorous environmental conditions, such as, at more exposed sites, at higher tidal levels, or under Arctic conditions.

Authentic specimens examined: **West Greenland**. Godthaab, July 1886, *K. Rosenvinge* (BM, *Plantae groenlandicae*, No. 211, ex Herb. Holmes). **Canada**. NOVA SCOTIA: Halifax harbour, Aug.–Sept. 1948, *T. A. & A. Stephenson* (Herb. M. S. Doty, No. NSH. 13). BRITISH COLUMBIA: Vancouver Island: Esquimalt, Jan. 1860, probably *F. S. Collins* (BM, ex Herb. Holmes, two sheets). **U.S.A.** MASSACHUSETTS: Nahant, Aug. 1884, *F. S. Collins* (BM, ex Herb. Holmes).

OTHER DESCRIBED FORMS

Apart from the forms reduced to synonymy in the foregoing treatment, certain other described forms have been regarded as distinct species by some recent authors, as follows:

Fucus fueci De la Pyl. (A. D. Zinova, 1953).
Fucus miclonensis De la Pyl. (Taylor, 1937; A. D. Zinova, 1953).
Fucus membranaceus Gardner (1922; Setchell & Gardner, 1925).

These forms are regarded by the author as further forms of *F. distichus* L. emend. Powell, but they do not appear to be sufficiently distinctive to warrant subspecific status.

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In conclusion, the author would be grateful to receive or borrow specimens of this difficult group of plants from colleagues in any part of the world, and especially from colleagues who may not agree with the taxonomic treatment proposed above.

SUMMARY

The taxonomic status and nomenclature of the hermaphrodite forms of *Fucus* (other than the alga currently known as *F. spiralis* L. and certain hybrid forms) usually included under the name *Fucus inflatus* L. by European authors, but under several specific names by N. American and some other authors, is discussed. It is considered that the numerous forms described are best interpreted for the present as forms of a single, extremely plastic and widely distributed species.

In order to determine the correct names to apply to two forms of '*F. inflatus* L.' found in Britain, a critical study has been made of the type specimens of *Fucus* spp. in the Linnaean Herbarium. It is concluded that the two small sterile specimens labelled '*F. inflatus*' in Herb. Linn. are most probably plants of *F. vesiculosus* L.; the original diagnosis of *F. inflatus* L. is also inadequate and it is proposed that this name be regarded as a synonym of *F. vesiculosus* L.

However, the taxon *F. distichus* L. (*Syst. Nat.*, Ed. 12, 1767) is shown to be valid, based on good specimens in Herb. Linn., and this is re-established as the earliest acceptable name for the group of forms in question. The principal characters of the species as a whole are: (i) hermaphrodite conceptacles, and (ii) the frequent presence, in all forms, of closed cavities in the frond—the caecostomata. Caecostomata are described for European material (including the Linnaean type) for the first time.

Near the centres of distribution of this species whole series of forms

(clines) intermediate in character between the various extreme forms may be found; in such places the extreme forms can often be interpreted as ecological forms and it may be that in some places they have evolved into true ecotypes (genetically better adapted forms). At the limits of geographical range, on the other hand, intermediate forms are often absent and only one or two of the principal forms may persist, often as populations isolated both geographically and ecologically; it is even more likely that these isolated populations are genetically distinct ecotypes, but this could only be proved by experimentation.

The taxonomic limits of *F. distichus* L. are revised to include the following four principal subspecies: subsp. *distichus*; subsp. *anceps* (Harv. et Ward ex Carruthers) Powell, *n.comb.*; subsp. *edentatus* (De la Pyl.) Powell, *n.comb.*; and subsp. *evanescens* (C. Ag.) Powell, *n.comb.* Of these, only subspp. *anceps* and *edentatus* have been found in Britain.

For each subspecies the following information is given: important synonymy, nomenclatural type, principal illustrations, description, geographical distribution, and a list of authentic specimens examined. The illustrations include photographs of the type specimens of *F. inflatus* L. and *F. distichus* L.

It is shown, incidentally, that the plant currently known in Britain as *F. vesiculosus* f. *evesiculosus*, should more properly be named *F. vesiculosus* f. *linearis* (Huds.).

REFERENCES

AGARDH, C. A., 1820. *Species algarum...* Vol. 1, Pt. 1, 168 pp. Lund.

—— 1821. *Icones algarum ineditae.* Fasc. 2, 2 pp., pls. 11–20. Stockholm.

AGARDH, J. G., 1868. Bidrag till Kännedomen af Spetsbergens Alger. Tillägg till föregående afhandling. *K. svenska VetenskAkad. Handl.*, Bd. 7, No. 8, pp. 27–49, pl. 3.

ANON., 1863. *Fucus furcatus* Agardh, a new British seaweed. *J. Bot., Lond.*, Vol. 1, p. 283.

ARESCHOUG, J. E., 1868. Slägtena *Fucus* (L.) Decaisne & Thuret och *Pycnophycus* Kütz. jemte tillhörande arter. *Bot. Notiser, 1868*, No. 3, pp. 99–115.

ARWIDSSON, TH., 1937. Meeresalgen aus Vestagder und Rogaland. *Nyt. Mag. Naturv.*, Bd. 76, pp. 85–149.

BATTERS, E. A. L., 1902. A catalogue of the British marine algae...*J. Bot., Lond.*, Vol. 40, Suppl., 107 pp.

BÖRGESEN, F., 1902. Marine algae. *Botany of the Faeröes*, Part II, pp. 339–532, pls. XIII–XXIV. Copenhagen, 1903. (Issued separately.)

—— 1903. The marine algae of the Shetlands. *J. Bot. Lond.*, Vol. 41, pp. 300–6.

—— 1904. *Om Algevegetationen ved Faeröernes Kyster, en Plantegeografisk Undersögelse.* 122 pp., 12 pls. Copenhagen.

—— 1905. The algae-vegetation of the Faeröese coasts, with remarks on the phytogeography. *Botany of the Faeröes*, Part III, pp. 683–834. Copenhagen, 1908. (Issued separately.)

BURROWS, E. M., CONWAY, E., LODGE, S. M. & POWELL, H. T., 1954. The raising of intertidal algal zones on Fair Isle. *J. Ecol.*, Vol. 42, pp. 283–8.

CARRUTHERS, W., 1863. *Fucus distichus* Linn. as an Irish plant. *J. Bot., Lond.*, Vol. 1, pp. 353–5, pl. 12.

—— 1864. Note on the Kilkee *Fucus*. *J. Bot., Lond.*, Vol. 2, p. 54.

Decaisne, J. & Thuret, G., 1845. Recherches sur les anthéridies et les spores de quelques *Fucus*. *Ann. Sci. nat.*, Sér. 3, Bot., T. 3, pp. 5–15, pls. 1–2.

De la Pylaie, A. J. M. B., 1829. *Flora de Terre-Neuve et des iles Saint Pierre et Miclon.* 128 pp. Paris. [The plates of this work were never published.]

De Toni, J. B., 1895. *Sylloge Algarum omnium hucusque cognitarum.* Vol. III, *Fucoideae*, 638 pp. Patavia.

Doty, M. S., 1947. The marine algae of Oregon. Part 1. Chlorophyta and Phaeophyta. *Farlowia*, Vol. 3, pp. 1–65.

Dunn, M. D., 1937. Notes on the flora of Loch Harray and Loch Stenness.... *Trans. bot. Soc. Edinb.*, Vol. 32, Pt. 2, pp. 368–72.

Du Rietz, G. E., 1947. Wellengrenzen als ökologische Äquivalente der Wasserstandslinien. *Zool. Bidr. Uppsala*, Bd. 25, pp. 534–50, 4 pls.

Esper, E. J. C., 1800. *Icones Fucorum*... Erster Theil, Viertes Heft, pp. 167–217 [including many erroneous page numbers], pls. 88–111. [This reference not seen; obtained from Silva, 1953.]

Farlow, W. G., 1881. *The Marine Algae of New England and Adjacent Coast.* Reprinted from *Rep. U.S. Comm. Fish.* for 1879, Appendix A–1, 210 pp., 15 pls. Washington.

'*Flora Danica.*' *Icones Plantarum sponte nascentium in regnis Daniae et Norwegiae, in ducatibus Slesvici et Holsatiae, et in comitatibus Oldenburgi et Delmenhorstiae: ad illustrandum opus de iisdem Plantis, Regio jussu exarandum, Florae Danicae nomine inscriptum.* [Usually quoted as 'Flora Danica'.] Edited at first by G. C. Oeder, later by others. 17 vols. (and 1 vol. Supplement), 3060 (and 180) pls., with descriptive letterpress. Hafniae, (1761–) 1764–1883. (See also Oeder, 1767, and Vahl, 1794.)

Foslie, M., 1886. Kritisk fortegnelse over Norges havsalger efter aeldre botaniske arbeider indtil aar 1850. *Tromsø Mus. Aarsh.*, Vol. 9, pp. 85–137.

Fritsch, F. E., 1945. *The Structure and Reproduction of the Algae.* Vol. 2, 939 pp. Cambridge University Press.

Gardner, N. L., 1922. The genus *Fucus* on the Pacific coast of North America. *Univ. Calif. Publ. Bot.*, Vol. 10, No. 1, 181 pp., incl. 60 pls.

Gauld, D. T., Bagenal, T. B. & Connell, J. H., 1953. The marine fauna and flora of St Kilda, 1952. *Scot. Nat.*, Vol. 65, pp. 29–49.

Gmelin, S. G., 1768. *Historia Fucorum.* 239 pp., 34 pls. Petropoli.

Gray, S. O., 1867. *British Sea-Weeds*, xxiii, 312 pp., 16 pls. col. London.

Harvey, W. H., 1864. *Fucus anceps* Ward & Harvey. A letter in *Ann. Mag. nat. Hist.*, 3rd. Ser., Vol. 13, p. 184.

Hornemann, J. W., 1827. *Nomenclatura Florae Danicae, emendata cum indice systematico et alphabetico.* 214 pp. Hafniae.

Hudson, W., 1762. *Flora Anglica.* xv, 506 pp. London.

—— 1778. *Flora Anglica.* Ed. 2, xxxviii, 690 pp. London.

Hygen, G. & Jorde, I., 1935. Beitrag zur Kenntnis der Algenflora der norwegischen Westküste. *Bergens Mus. Aarb.* 1934, Nr. 9, 60 pp.

Hylmö, D. E., 1933. Algenimmigration nach der schwedischen Westküste. *Bot. Notiser*, 1933, pp. 377–90.

Inoh, S., 1935. Embryological studies on *Pelvetia wrightii* Yendo and *Fucus evanescens* Ag. *J. Fac. Sci. Hokkaido Univ.*, Ser. V, Bot., Vol. V, No. 1, pp. 9–23, 4 pls.

Jónsson, H., 1903. The marine algae of Iceland. II. Phaeophyceae. *Bot. Tidsskr.*, Bd. 25, pp. 141–95.

Kjellman, F. R., 1883. The algae of the Arctic Sea. *K. svenska VetenskAkad. Handl.*, Bd. 20, No. 5, 350 pp., 31 pls.

KJELLMAN, F. R., 1890. *Handbok i Skandinaviens Hafsalgflora.* I. *Fucoideae.* 103 pp. Stockholm.

KLEEN, A. E., 1874. Om Nordlandens högre hafsalger. *Öfvers. VetenskAkad. Förh., Stockh.*, No. 9, pp. 3–46, pls. 9, 10.

KÜTZING, F. T., 1860. *Tabulae Phycologicae, oder Abbildungen der Tange.* Bd. 10, 39 pp., 100 pls. Nordhausen.

KYLIN, H., 1947. Die Phaeophyceen der schwedischen Westküste. *Lunds Univ. Årsskr.*, N.F., Avd. 2, Bd. 43, Nr. 4, 99 pp., 18 pls.

LANJOUW, J. (Editor) *et al.*, 1952. *International Code of Botanical Nomenclature... Adopted... Stockholm, July 1950.* 228 pp. Utrecht.

LEVRING, T., 1937. Zur Kenntnis der Algenflora der norwegischen Westküste. *Lunds Univ. Årsskr.*, N.F., Avd. 2, Bd. 33, Nr. 8, 148 pp., 4 pls.

—— 1946. Några havsalgfynd vid den svenska västkusten. *Acta Hort. gothoburg.*, 16, pp. 191–4.

LEWIS, J. R., 1954. The ecology of exposed rocky shores of Caithness. *Trans. roy. Soc. Edinb.*, Vol. 62, pp. 695–723, 4 pls.

LIGHTFOOT, J., 1777. *Flora Scotica.* 2 Vols. London.

LINNAEUS, C., 1737. *Flora Lapponica.* Ed. 1. Amstelaedami.

—— 1753. *Species Plantarum.* Ed. 1, 2 T. Holmiae.

—— 1767. *Systema Naturae.* Ed. 12, 3 T. Holmiae, 1766–68. (T. 1, 1766; T. 2, 1767; T. 3, 1768.)

—— 1774. *Systema Vegetabilium.* Gottingae and Gothae. [This is a new edition of the botanical part (T. 2) of the *Systema Naturae* and counts on from the 12th edition (1766–68) of that work.]

—— 1779. *Systema Plantarum.* Francofurti and Moenum.

LUND, S., 1949*a*. Nye Alger for de danske Farvande. (With summary in English.) *Bot. Tidsskr.*, Bd. 48, Hft. 3, pp. 231–53.

—— 1949*b*. Immigration of algae into Danish waters. *Nature, Lond.*, Vol. 164, p. 616.

LYNGBYE, H. C., 1819. *Tentamen Hydrophytologiae Danicae.* 248 pp., 70 pls. Hafniae.

NEWTON, L., 1931. *A Handbook of the British Seaweeds.* 478 pp. London.

NICHOL, E. A. T., 1938. The brackish-water lochs of Orkney. *Proc. roy. Soc., Edinb.*, Vol. 58, pp. 181–91.

NORUM, E., 1913. Brunalger fra Haugesund og Omegn. *Nyt. Mag. Naturv.*, Bd. 51, pp. 137–60, pl. 2.

OEDER, G. C. (Editor), 1767. In '*Flora Danica*' [q.v.], Vol. 2, fasc. 6, tab. 351.

PARKE, M., 1953. A preliminary check-list of British marine algae. *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 497–520.

POWELL, H. T., 1954. The distribution, ecology and taxonomic status of forms of *Fucus inflatus* L. in Britain and Ireland. Summary in *Rapp. Comm., 8me Congr. Int. Bot., Paris 1954*, Section 17, pp. 135–6.

POWELL, H. T. & LEWIS, J. R., 1952. Occurrence of *Fucus inflatus* L. forma *distichus* (L.) Börgesen on the north coast of Scotland. *Nature, Lond.*, Vol. 169, p. 508.

PRINTZ, H., 1926. Die Algenvegetation des Trondhjemsfjordes. *Skr. norske Vidensk-Akad.*, I Mat.-Naturv. Klasse, No. 5, 274 pp., 10 pls.

ROSENVINGE, L. K., 1893. Grønlands Havalger. *Medd. Grønland*, Bd. 3, pp. 763–981, 2 pls.

SAVAGE, S., 1945. *A Catalogue of the Linnaean Herbarium.* 226 pp. London.

SETCHELL, W. A. & GARDNER, N. L., 1925. The marine algae of the Pacific coast of North America. Part III, Melanophyceae. *Univ. Calif. Publ. Bot.*, Vol. 8, Part 3, pp. 383–898, incl. pls. 34–107.

SILVA, P. C., 1953. The identity of certain Fuci of Esper. *Wasmann J. Biol.*, Vol. 11, No. 2, pp. 221–32.

SMITH, G. M., 1944. *Marine Algae of the Monterey Peninsula California.* 622 pp., incl. 98 pls. Stanford University Press, California; Oxford University Press.

SUNDENE, O., 1953. The algal vegetation of Oslofjord. *Skr. norske VidenskAkad.*, I Mat.-Naturv. Klasse, No. 2, 244 pp., 1 end-map.

TAYLOR, W. R., 1937. Marine algae of the northeastern coast of North America. *Univ. Mich. Stud.*, Vol. 13, 427 pp., 59 pls.

TURNER, D., 1802. *A Synopsis of the British Fuci.* 400 pp. Yarmouth.

—— 1808. *Fuci*... Vol. 1. London.

—— 1809. *Fuci*... Vol. 2. London.

VAHL, M. (Editor), 1794. In '*Flora Danica*' [q.v.], Vol. 7, fasc. 19, tab. 1127.

YENDO, K., 1907. The Fucaceae of Japan. *J. Coll. Sci. Tokyo*, Vol. 21, No. 12, 174 pp., 18 pls.

ZINOVA, A. D., 1953. *Opredelitel' burykh vodoroslei severnykh morei SSSR.* [*Title transl. : Classification of brown algae of the northern seas of the U.S.S.R.*] 225 pp. (153 illus.) Bot. Inst., Acad. Sci., U.S.S.R. Moscow & Leningrad. [In Russian.]

—— 1954. [*Title transl.:* Notes for Flora of seaweeds found in the White Sea.] *Acta Inst. bot. Acad. Sci. URSS*, Ser. 2, Plantae Cryptogamae, Fasc. 9, pp. 245–58. [In Russian.]

ZINOVA, E. S., 1933. Vodorosli Kamchatki. [Algae of Kamchatka]. *Issledovaniia morei S.S.S.R.*, Fasc. 17, pp. 7–42. [In Russian, with short summary in French.]

—— 1954. [Seaweeds found (*a*) in Okhotsk Sea, (*b*) in the Gulf of Tartary, (*c*) round south-eastern Kamchatka (*Titles transl.*).] *Acta Inst. bot. Acad. Sci. URSS*, Ser. 2, Plantae Cryptogamae, Fasc. 9, pp. 259–310, 311–64, 365–400. [In Russian.]

EXPLANATION OF PLATES

PLATE I

Fig. 1. *Fucus inflatus* L. Photograph of the type specimens (Sheet 1274: 51 in the Linnaean Herbarium).

Fig. 2. *F. distichus* L. emend. Powell, subsp. *distichus*. Photograph of the type specimens (Sheet 1274: 56 in the Linnaean Herbarium). Specimen A is the lectotype.

PLATE II

Fig. 1. *F. distichus* L. emend. Powell, subsp. *anceps* (Harv. et Ward ex Carruthers) Powell. Photograph of a pressed specimen, 11·5 cm greatest length: Kilkee, Ireland, *J. Cook* (BM, ex Herb. Holmes, no date). × 0·55.

Fig. 2. *F. distichus* L. emend. Powell, subsp. *edentatus* (De la Pyl.) Powell. Photograph of a pressed specimen, 25 cm greatest length: Lerwick, Shetland Islands, July 1952, *H. T. Powell* (MILL). × 0·35. *r.*, elongated receptacles; *c.*, continued vegetative growth beyond the receptacles. The receptacles are not usually so much elongated as in this specimen.

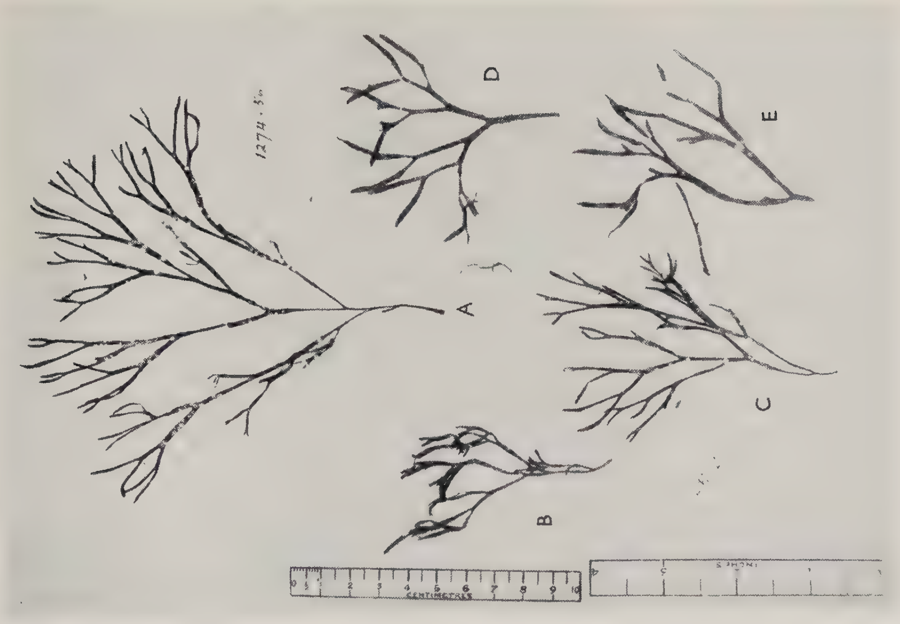

1274·50
A
B
C
D
E
CENTIMETRES
INCHES

Fig. 2

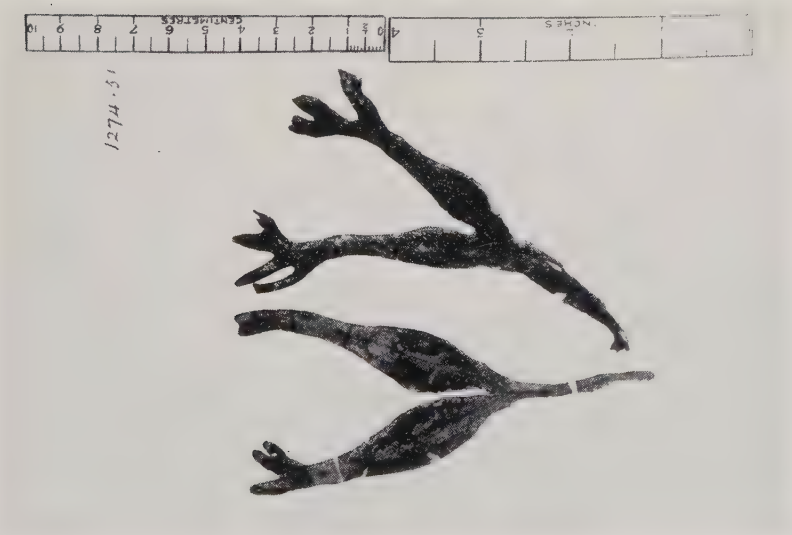
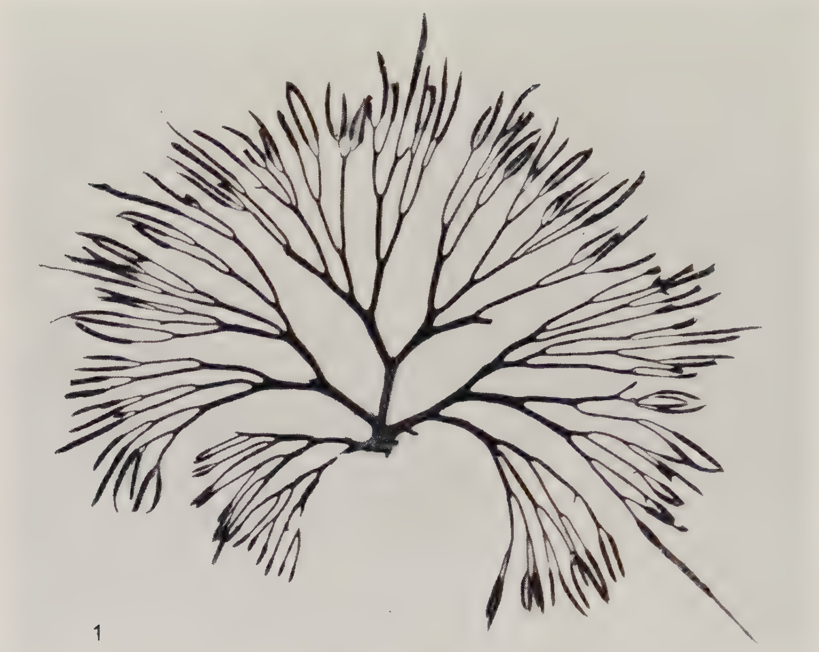
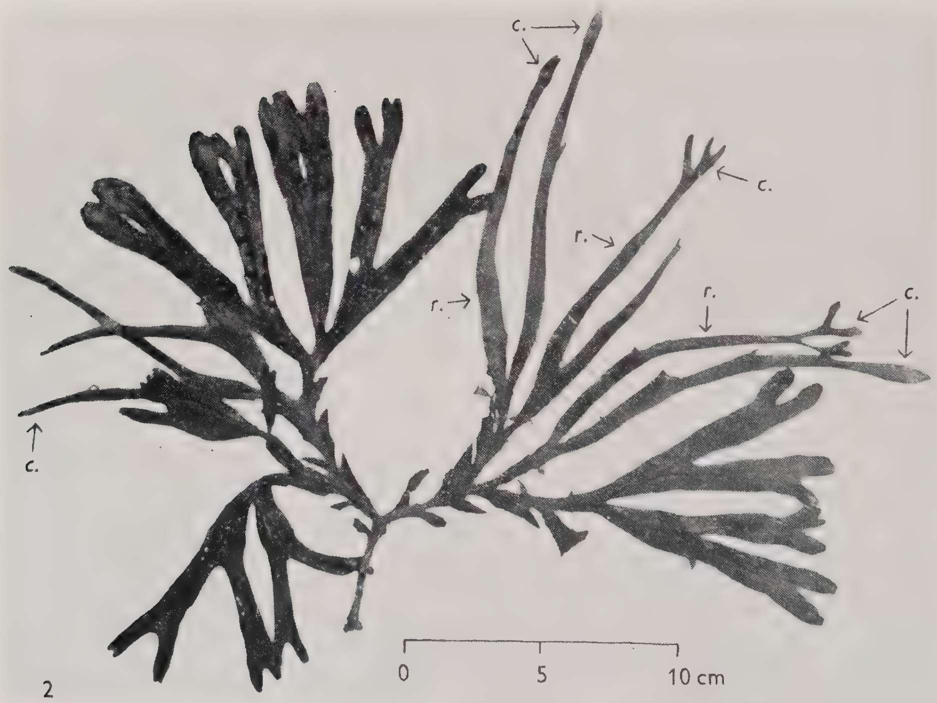

1274·51
CENTIMETRES
INCHES

Fig. 1

(*Facing p.* 432)



1



c.
c.
c.
r.
r.
r.
c.
c.
0
5
10 cm

2

ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

NOTES ON THE NERVOUS SYSTEM IN THE STOMATOPODA. IV. MUSCLE RECEPTOR ORGANS

By J. S. Alexandrowicz

Pubbl. Staz. Zool. Napoli, 1954, Vol. 25, pp. 94–111

In *Squilla mantis*, muscle receptor organs have been found in the extensor muscles of the six thoracic and six abdominal segments. They are composed of thin muscles and nerve cells. In ten segments there are two muscles and two nerve cells on each side. The muscles of each pair differ in their structure. In a certain region of the muscles there is an intercalated tendon common to the two muscles. The nerve cells differ in their size and mode of ramification of their dendrites.

Differences in structure of the muscle receptor organs which might have a relation to their response at different stretch thresholds are discussed.

J.S.A.

THE EYES AND THE PHOTONEGATIVE BEHAVIOUR OF *NEPHTYS* (ANNELIDA, POLYCHAETA)

By R. B. Clark

J. exp. Biol., 1956, Vol. 33, pp. 461–77

Three types of photoreceptor are found in the Nephtyidae: inverted, single-celled ocelli in pigment cups embedded in the brain, similar cells, though without pigment cups, anterior to the brain, and possibly epidermal photoreceptors. Morphological and experimental evidence suggests that the prostomial receptors of *Nephtys* are homologous with the eyes of *Nereis* and that they are involved in the same types of behaviour. The frequency with which *Nephtys* swims is, within limits, a linear function of the light intensity. Although the ganglionic eyes are directional receptors the worm does not orientate itself in a light beam; presumably the light reaching them is too diffuse. In the very small species *N. cornuta* the eyes are close to the surface of the brain and the worm does orientate itself in a light beam. Swimming is an essential prelude to burrowing, and the brighter the light the more frequently the worm swims and the sooner it is buried. Activity in light can be inhibited by stimulating receptors on the dorsal surface of the animal by contact.

R.B.C.

DEEP WATER MOVEMENTS IN THE NORTH ATLANTIC AS A LINK BETWEEN CLIMATIC CHANGES AROUND ICELAND AND BIOLOGICAL PRODUCTIVITY OF THE ENGLISH CHANNEL AND CELTIC SEA

J. mar. Res., 1955, Vol. 14, pp. 347–62

AND

HYPOTHESES CONNECTING FLUCTUATIONS IN ARCTIC CLIMATE WITH BIOLOGICAL PRODUCTIVITY OF THE ENGLISH CHANNEL

By L. H. N. Cooper

Deep-Sea Res., 1955, Suppl. to Vol. 3, pp. 212–23

These two papers, which need to be read together with the paper in the *Journal* on assessing the age of oceanic water with carbon-14, present a group of hypotheses to explain fluctuations in biological productivity of the English Channel, in terms of changes in Arctic climate. The first paper describes the conditions under which heavy and cold Norwegian Sea water spills over the ridges east and west of Iceland into the Atlantic to create two new water masses. The courses of these are followed down into the deep Atlantic. In each case the water is held to the right banked against the eastern side of the Reykjanes Ridge and the continental slope of East Greenland respectively. Room in the ocean for this sinking water has to be found by upward displacement of water which is already there. This upward displacement will carry the deep stock of nutrients towards the upper layers where wind-driven and thermohaline mixing processes—which are always operating—may bring them into the illuminated zone. When cold Arctic winters cause much recruitment of cold heavy water to the Norwegian Sea, a chain of events is initiated which will enrich the surface waters. Following mild Arctic winters the loss from the surface layers in temperate latitudes by sinking of detritus and defecation at depth of vertical migrants, cannot be made good by upward displacement.

This, however, is only one part of the mechanism. The second paper develops a complementary one.

The overspills into the Atlantic are believed to occur not as smooth steady streams of water but as intermittent surges. In the Atlantic these surges will behave as balls or boluses of water and may be considered to have some of the properties of an elastic solid. Whilst sinking beneath the lighter enveloping water and especially when the under-lying sea-bed or continental slope is very dissected, the boluses may initiate internal waves of considerable amplitude and energy. It is postulated that these waves may travel great distances in the ocean with little attenuation. When they strike another continental slope, particularly a dissected one, much of the energy of the internal wave system is likely to be destroyed by mixing of the waters bathing the slope. The resulting homogenization of these slope waters will tend to carry nutrients upwards and heat downwards. This chain of events will also be favoured by cold Arctic winters and weakened by mild ones.

From the basic hypotheses it is easy to derive further ones which are capable of test by observation and experiment in the ocean.

L.H.N.C.

DEMONSTRATION OF SMALL-SCALE WATER-CURRENTS BY MEANS OF MILK

By D. J. Crisp and A. J. Southward

Nature, Lond., 1956, Vol. 178, p. 1076

During work on the behaviour of barnacles it was found that ordinary cow's milk offered a better means of detecting small-scale water movements than the commonly used suspensions of carmine or graphite. The animals were observed or photographed against a black background and the milk was injected into the water above from a row of fine jets. The density of the milk could be varied by dilution with the water or by alteration of the cream content; normally it remained suspended in trails, resembling thick smoke in air. The method was used to measure the water currents set up by the cirral movements of barnacles, and to establish the presence of a through current in the mantle cavity during normal beating of the cirri.

A.J.S.

LA CONTAMINATION DES POISSONS ET LE PROBLÈME DES EAUX POLLUÉES

By A. Guelin

Annales Inst. Pasteur, 1954, T. 86, pp. 303–9

It has been previously reported by the writer that the intestine of fishes caught offshore in the Mediterranean is free from enterobacteria, whereas in those caught in the littoral zone there is an intestinal flora similar to that in man or in warm-blooded animals.

The waters sampled far from any shore are as a rule free from bacteria and intestinal bacteriophages found in the littoral zone. The question arises how far the bacteriological aspect of the viscera of fishes is correlated with the sanitary state of waters in which they live.

Sixty-one specimens of *Ctenolabrus rupestris*, in which no infestation could be ascertained in preliminary examination, were contaminated with either *Enteramoeba coli* or with coliphage. In a week's time all these fish had freed themselves completely from the contaminating agent.

These results show that the investigation of the enterobacteria and enterophages in fishes could give information as to the possible pollution of the waters in which the animals live.

A.G.[1]

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[1] This abstract has been translated from the French.

STUDIES OF SERPULID TUBE FORMATION.

II. THE CALCIUM-SECRETING GLANDS IN THE PERISTOMIUM OF *SPIRORBIS, HYDROIDES* AND *SERPULA*

By R. H. Hedley

Quart. J. micr. Sci., 1956, Vol. 97, pp. 421–7

In a previous paper the author described the calcium-secreting glands in *Pomatoceros* and gave an account of the pre-secreted component of this serpulid's tube. This study has been extended to three other British serpulids and the structural variation of the calcium-secreting glands has been demonstrated.

In *Spirorbis* the gland is tubular, in which the gland cells have unusual extensions projecting into the lumen, whilst in *Hydroides* the gland is a simple tubule. *Serpula* has glands which are tubulo-racemose and similar to those found in *Pomatoceros*.

An unusual arrangement is found in *Serpula* where, in addition to the two calcium-secreting glands, there are two ventral calcium sacs in the posterior part of the peristomium. These also secrete calcareous material and contribute towards tube formation. R.H.H.

ACTIVE TRANSPORT OF CATIONS IN GIANT AXONS FROM *SEPIA* AND *LOLIGO*

By A. L. Hodgkin and R. D. Keynes

J. Physiol., 1955, Vol. 128, pp. 28–60

In giant axons from *Sepia* and *Loligo* the efflux of labelled sodium ions and the influx of potassium ions have been shown to be greatly reduced by metabolic inhibitors (dinitrophenol, cyanide and azide) or by cooling to a low temperature. Sodium influx and potassium efflux are relatively little changed under these conditions. Metabolic inhibitors also have little effect on the resting potential and action potential, or on the rapid sodium movements associated with the passage of impulses. Removal of potassium ions from the external medium reduces the sodium efflux to about one-third, the effect being immediate in contrast to the delayed action of inhibitors, and the absolute size of the decrease in efflux being about equal to the decrease in potassium influx caused by inhibitors.

It is concluded that in addition to a permeability system which allows ions to move down electrochemical gradients during electrical activity, there is a secretory mechanism driven by metabolism which ejects sodium and absorbs potassium against the electrochemical gradients. Conduction of impulses, but not recovery, can take place if the secretory mechanism is put out of action with inhibitors. Sodium efflux and potassium influx are coupled, but do not seem to be linked rigidly.

R.D.K.

EXPERIMENTS ON THE INJECTION OF SUBSTANCES INTO SQUID GIANT AXONS BY MEANS OF A MICROSYRINGE

By A. L. HODGKIN and R. D. KEYNES

J. Physiol., 1956, Vol. 131, pp. 592–616

A microsyringe is described which enables precisely determined volumes of fluid to be injected into squid giant axons uniformly over distances of 3–20 mm. The volume injected is about 1/25 of the axon volume per unit length. The performance of the microsyringe was tested by injecting dye solutions. Methylene blue and eosin were observed to diffuse radially through the axoplasm, but more slowly than in free solution.

Injection of small quantities of KCl did not have any marked effect on the membrane potentials. Injection of similar quantities of NaCl reduced the reversed potential at the crest of the spike by an amount which fitted with that calculated from the change in concentration ratio. Injections of magnesium and tubocurarine chlorides had no great effect on the axons. Injections of calcium liquefied the axoplasm and tended to block conduction.

When ^{24}NaCl was injected, a steady rate of extrusion of labelled sodium was established within a minute. The diffusion coefficient of sodium in the axoplasm can have been very little less than in free solution, and the sodium pump must operate with a lag of not more than a few seconds. The sodium efflux was blocked in the usual way by inhibitors. It was established that over short periods the sodium efflux was directly proportional to the internal sodium concentration, but that the proportionality factor declined with a time constant of about 5 h.

R.D.K.

TEMPERATURE AND MACKEREL MOVEMENTS IN THE INSHORE WATERS OF TORBAY, DEVONSHIRE

By L. A. J. JACKMAN and G. A. STEVEN

J. Cons. int. Explor. Mer, 1955, Vol. 21, pp. 65–71

The arrival and departure date of mackerel in the Torquay area show a close correlation with sea surface temperature.

In the period 1945–53 inclusive mackerel made their first appearance inshore when the sea temperature was within the range 11·1–11·6° C. In six different years the temperature was exactly 11·6° C and on those occasions when it differed a reading of 11·6° C was made either one day before or one day after the recorded appearance of the fish. Departure records for the years 1941 to 1953 show a range of 12·2°–13·9° C.

In no year did the fish arrive before a well-marked thermocline had formed at E_1. Conversely, on departure the thermocline had almost always broken down completely.

This fact will be more closely observed in future years. L.A.J.J.

SEASONAL CHANGES IN THE PHYTOPLANKTON AS INDICATED BY SPECTROPHOTOMETRIC CHLOROPHYLL ESTIMATIONS 1952–53

By Pamela G. Jenkins

Deep-Sea Res., 1955, Suppl. to Vol. 3, pp. 58–67

Chlorophyll determinations were continued on the phytoplankton of the English Channel from September 1952 until August 1953 at depths from 0 to 70 m, the species being identified by culture. Minima 2 mg/m^3 occur in winter and June. Maxima at particular depths can occur in March, April or May. In 1952, 34·2 mg/m^3 was a maximum in the surface, whereas in 1953 cell sinking gave, in May, 78·8 mg/m^3. The autumn maximum in September 1952 was 21·1 mg/m^3.

Collodion filter disks varied from dark grey or chocolate to a light sandy colour and showed phytoplankton, fibres, copepods and other animals. Copepods were about 300,000 per square metre column down to 70 m in April.

Fifty-four species of Bacillariophyceae were recorded. As before *Skeletonema costatum*, a Navicula and *Nitzschia closterium* were the most common. Six Chlorophyceae, five Chrysophyceae, one Cyanophyceae and three Cryptophyceae were found. In the first class a *Chlorella* sp. was commonest and in the second a species of Coccolithophora grew in each sample. *Phaeocystis globosa* grew from January to May. *Hemiselmis rufescens* appeared again.

P.G.J.

COLLOIDAL PROPERTIES OF THE MESOGLOEA IN SPECIES OF *LEUCOSOLENIA*

By W. C. Jones

Quart. J. micr. Sci., 1956, Vol. 97, pp. 269–85

Isotonic potassium nitrate solution causes a rapid collapse of the oscular tubes of species of *Leucosolenia*: the cells dissociate and the mesogloea swells and disperses. Differences in the time of collapse of tubes from different specimens, or tubes derived at different times from the same specimen, suggest that the mesogloea varies in its initial degree of firmness. Replacing the collapsing tubes in sea water results in the immediate stiffening of the mesogloea and the previously swollen cells shrink and form characteristic rod-like processes. The mesogloea seems to be secreted by the choanoderm, since just beneath this layer it may be more readily swollen by the potassium nitrate. Its dispersion can be prevented by first immersing the tubes for a time in slightly acidified sea water. Other neutral salt solutions also soften the mesogloea, but vary considerably in the time taken for the onset of plasticity. Their ions can be arranged in three lyotropic series which indicate that the mesogloea is an organic hydrophilic colloid similar to, for example, chondroitin sulphate.

The experiments show that the mesogloea has an important skeletal function.

W.C.J.

THE INTRACELLULAR CALCIUM CONTENTS OF SOME INVERTEBRATE NERVES

By R. D. Keynes and P. R. Lewis

J. Physiol., 1956, Vol. 134, pp. 399–407

A method is described for the determination in biological samples of 1 μg or less of calcium. Analyses of (*a*) axoplasm extruded from freshly dissected squid axons, and (*b*) whole *Carcinus* nerves which had been soaked in Ringer's solution containing various amounts of calcium, showed that in both types of invertebrate nerve the internal calcium concentration is of the order of 0·5 m-mole/kg wet weight.

R.D.K.

EVIDENCE FOR A MECHANORECEPTIVE FUNCTION OF THE AMPULLAE OF LORENZINI

By R. W. Murray

Nature, Lond., 1957, Vol. 179, pp. 106–7

The responses of the ampullae of Lorenzini of *Raia clavata* were investigated electrophysiologically. The spontaneous frequency of discharge of single units could be increased or decreased by stimuli which increased or decreased the pressure within the ampullae themselves relative to that outside. The responses showed partial adaptation and opposite after-effects. Possible functions of the ampullae are discussed.

R.W.M.

PHYSIOLOGICAL CONTROL OF LUMINESCENCE IN ANIMALS

By J. A. C. Nicol

Luminescence of Biological Systems, pp. 299–321. 1955. Washington: American Ass. Adv. Sci.

A review is presented of various methods known to be operative in the regulation of the luminescent responses of animals. Intensity, duration and iteration are possible variables. Control is direct (excitation acting directly on intracellular photogenic processes), or indirect, involving glandular secretion (extracellular luminescence) or movement of screening devices (symbiotic organs).

Various examples are given, and some new data presented for luminescence in ctenophores, the glow-worm and Pyrosoma. J.A.C.N.

THE NERVOUS ANATOMY OF THE BODY SEGMENTS OF NEREID POLYCHAETES

By J. E. Smith

Phil. Trans. B, 1957, Vol. 240, pp. 135–96

An account is given of the anatomy of the giant and fine fibre systems of the nerve cord of nereid polychaetes and of the constitution of the four pairs of serially repeated segmental nerves.

The fine fibre internuncial systems are designed to transmit excitation ventro-dorsally in the cord and (by means of six longitudinal tracts) along its length. Most of the sensory and motor fibres effect their internuncial connexions within the cord in the dorsal half of the neuropilar substance. The nervous arcs which involve fine fibre internuncials are described in detail. They appear to have a more obviously direct nervous continuity than those involving giant fibres, a circumstance which is discussed in relation to previously established excitation characteristics of the two systems and their role in bringing about locomotory movements and 'escape' responses.

All the segmental nerves are 'mixed'. Their sensory fields and, in some instances, their motor distribution are described. There is, on the sensory side, anatomical evidence of the canalization of afferent excitation from a large number of widely separated sensory endings into a small number of centrally directed tracts. On the motor side the dispersal of excitation to the various muscle systems is brought about by the interpolation of relay neurons into the peripherally directed motor tracts which, at their origin from the cord, contain very few fibres. The possible significance of the small number of motor and internuncial neurons in the cord is discussed. J.E.S.

BOOK REVIEWS

THE GALATHEA DEEP SEA EXPEDITION

Described by members of the expedition
and edited by A. F. Bruun, S. Greve, H. Mielche and R. Spärck

Translated from the Danish by R. Spink.

George Allen and Unwin, London. 296 pp. Price 40*s*. 1957.

The Galathea Expedition, which set sail from Copenhagen in 1950, was only the fourth major expedition which was equipped to explore all the great deeps of the oceans. In two years she circumnavigated the world. When she departed very few dredge hauls had been made below 6000 and only one below 7000 m. It was seriously disputed whether life existed in the abyssal depths below 10,000 m. When she returned she had fished over 100 specimens, representing six species and five phyla, from depths of over 10,000 m and thousands more embracing all the major phyla, including vertebrates, from depths greater than any previously attempted. Living bacteria were brought up from the extreme depths, in mud samples, and cultured in the laboratory, where they would only grow under pressures of 1000 atmospheres. Any account of this expedition is inevitably exciting to anyone interested in marine biology, and this account is made doubly so by the freshness imparted by description given by those who took part. A book of this nature is, of course, patchy, since the various writers have very differing styles and concepts of their readers' backgrounds, but even this adds variety. The translation is perhaps a trifle clumsy. The illustrations are more than adequate and make quite certain that this will take its place among the books of permanent value devoted to the great explorations of the world.

D.B.C.

THE AMERICAN ARBACIA AND OTHER SEA URCHINS

By Ethel Browne Harvey

Published by Princeton University Press (London: Oxford University Press). Pp. i–xiv, 1–298, with 16 plates and 12 text-figures. Price 48*s*. 1956.

Echinoid genital products are to the invertebrate experimental embryologist almost what *Drosophila* is to the geneticist; without them he would be seriously handicapped and our knowledge of fertilization and cleavage would be less advanced than it is to-day. A vast literature has grown up around these processes as they occur in *Arbacia punctulata* of the eastern coast of North America, in *A. lixula* of the Mediterranean, in *Echinus esculentus* of our own shores and in other sea-urchins here and elsewhere. This book deals primarily with the first-named species, listing all important works about it, but including as well many works concerning other species. The book will be specially valuable to investigators at Woods Hole where the intensity of research into the embryology of *Arbacia punctulata* can be judged from the numbers of this sea-urchin used annually at the Marine Biological Laboratory there. Often several tens of thousands have been used in one year, though more recently less than ten thousand a year have sufficed. But the book will be scarcely less useful to those who work with the closely allied *Arbacia* at Naples and in other Mediterranean laboratories, and it cannot fail to be of very great value to embryologists using any species of sea-urchin. The text is delightfully written and apart from a few misprints the typography, by a Dutch firm, is good to the eye. The sequence of chapters takes us from the etymology of common and scientific names for the spiny adult urchins, their use as food for man and animals, through the classification and distribution of the different genera and species, to their embryology, normal and experimental. There are several chapters on centrifuged eggs, with which the author has herself done so much fundamental work. Most valuable of all is the compilation of notes on the experimental work of a formidable array of authors whose books and papers are listed in an extensive bibliography occupying over fifty two-columned pages. The sixteen beautifully reproduced plates, containing about 250 photomicrographs and other photographs of developing eggs, larvae and adults, merit praise. No zoological library and no worker in sea-urchin embryology and cell physiology can afford to be without this book.

D.P.W.

EELS: A BIOLOGICAL STUDY

By the late Léon Bertin

Published by the Cleaver-Hume Press Ltd., London. Pp. viii + 188 + 4.

Translated by Betty Roquerbe, under the supervision of Maurice Burton, from the second edition of *Les Anguilles* (Payot, Paris). Price 25*s*. 1956.

This curiously mixed book is the most complete and authentic account of the biology of the eel to appear in English and as such must take its place among the works of reference. All the information which the reader might require on freshwater eels (but not conger or Roman eels) is included, but often in a piecemeal and unassimilated manner. The book is evidently intended for the professional biologist or student, yet Professor Bertin occasionally felt it necessary to explain the most elementary biological principles as if he were writing for the lay reader. The bibliography has some strange omissions, notably Schmidt's paper, published in *Phil. Trans.* in

1922, in which he first announced the details of his discovery of the breeding place of eels. The half tone plates are poor, but the excellence of the line drawings compensates for this. While the factual content of the book is unimpeachable some of Professor Bertin's conclusions place too much credence on oceanographical theories of doubtful validity. His account of the larval migration of leptocephali is marred in this way and also suffers from an altogether needless attempt to convince the surely extinct sceptics of the truth of this migration. The account of the methods of statistical analysis of growth of eels is simultaneously both inadequate and unnecessary: it should have been omitted. The translation is well above average, but a few flaws have escaped revision.

D.B.C.

J. mar. biol. Ass. U.K. (1957) 36, 445–447
Printed in Great Britain

ON A NEW MEDUSA, *KRAMPELLA DUBIA* N.G., N.SP.

By F. S. Russell, F.R.S.
The Plymouth Laboratory

(Text-figs. 1 and 2)

In a collection made with a 2 m stramin ring trawl at 47° 03′ N., 5° 47′ W. on 4 July 1956, with 800 fathoms of wire out, I have found a new species of medusa. This specimen is in a comparatively good state of preservation except that the stomach is badly damaged.

The umbrella is hemispherical and the jelly is moderately thick. The form of the stomach cannot be described for certain. Parts of it are to be seen as narrow strips hanging down from the upper ends of three of the radial canals. One of these strips is continued for a short distance along the subumbrella surface towards the summit. It has the form of two short curtains with a space between them which leads into the radial canal. It thus seems quite possible that the stomach is in fact an open cross with mouth lips extending along each arm as in *Staurophora*.

The four radial canals are broad and there is a ring canal. Along each of the four radial canals there are about sixteen fine strands of tissue running through the jelly and connecting the walls of the radial canals with the exumbrella surface, similar to those I have recorded (Russell, 1956) in *Amphinema krampi*.

The gonads are situated along almost the whole length of each radial canal. They are widely divided longitudinally.

There are four perradial and four interradial marginal tentacles each with a swollen conical basal bulb. The tentacles coil spirally.

Between the bases of each pair of marginal tentacles there are three or four small marginal cirrus-like tentacles, the actual sequence being 3, 3, 4, 3, 4, 3, 4, 4.

No marginal vesicles, cordyli, or sense organs can be seen, and there are no ocelli apparent.

There is a little yellowish brown pigment on parts of the gonads and this colour is rather prominent on what appear to be the mouth lips. Otherwise the medusa is colourless. It is about 3 mm in diameter, and is a male.

A drawing of this medusa as seen from the subumbrellar side is given in Fig. 1 *a*. A lateral view, drawn slightly ideally, is given in Fig. 1 *b* in which the possible outlines of the stomach are indicated.

One quadrant of the medusa was removed for sectioning. Examination of these sections adds the following additional information.

The strands of the tissue running from the outer walls of the radial canals to the exumbrella surface consist of rows of cells which may be two or three thick in places (Fig. 2*a*). At some points there may be as many as three strands starting from approximately the same position on the radial canal, and

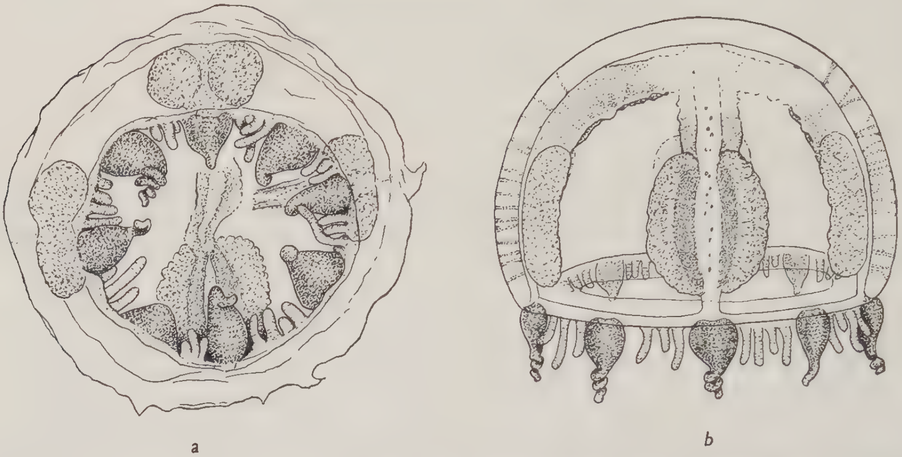

Fig. 1. *Krampella dubia* n.sp. *a*, viewed from subumbrellar side; *b*, lateral view slightly idealized, with suggested outlines of stomach.

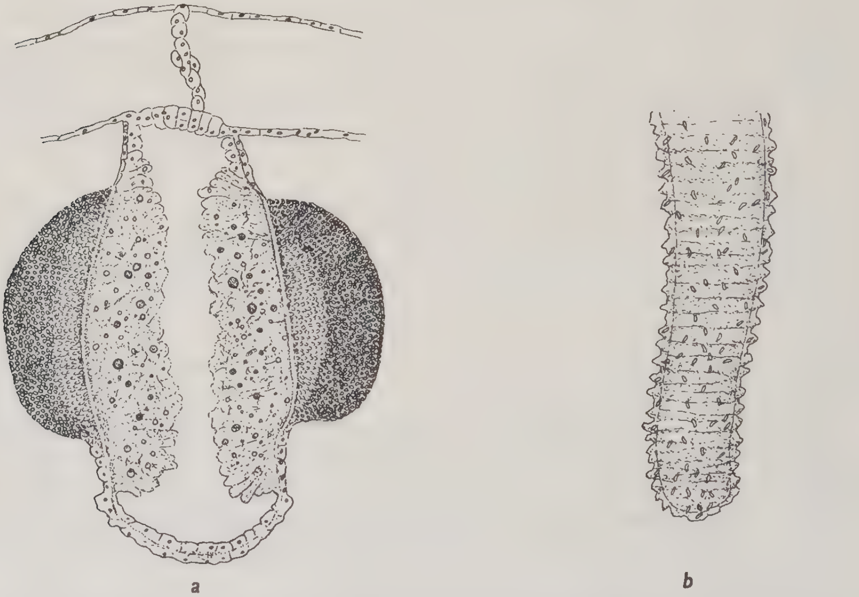

Fig. 2. *Krampella dubia* n.sp. *a*, section through radial canal and gonads, and showing strand of cells connecting wall of radial canal with exumbrella surface; *b*, marginal tentacula.

diverging to reach the exumbrella surface at three different spots. In no instance did there appear to be a definite canal surrounded by cells, though it is possible that there may be intercellular connexion of cavities between cells.

The radial canals carry the gonads on their lateral walls. They do not cover the whole of the walls dorso-ventrally, but begin a short distance from the subumbrella and are well separated ventrally (Fig. 2*a*). The endoderm of the greater part of the lateral wall consists of tall narrow digestive cells. Much of this tissue is in a disintegrated state, but in places the cell outlines can be seen.

The marginal tentacles are filled with cubical endoderm cells, and the basal bulbs and at any rate the proximal regions of the tentacles, are hollow.

The cirrus-like tentacles are cylindrical and hollow, having a single layer of cubical endoderm cells surrounding a central lumen. There are numerous small nematocysts evenly dispersed over them (Fig. 2*b*). They are evidently capable of extension since in their present state they are ringed with corrugations (Fig. 2*b*). From their structure they do not appear to be homologous with either cordyli or true marginal cirri which have a solid core of endoderm cells. It seems better to regard them as tentaculae or true tentacles of a different kind from the large tentacles.

I have examined the nematocysts, but not under oil immersion. There are two sizes; the larger, about 17 μ long undischarged, appeared to be an atrichous haploneme, and the smaller, about 9 μ long undischarged, a microbasic mastigophore. Both kinds were to be seen on the bases of the large marginal tentacles, but only the smaller kind on the tentaculae.

The systematic position of this medusa is problematical. It is evidently a Leptomedusa. The combination of characters, absence of marginal vesicles and possible cruciform shape of the stomach, might indicate affinities with the Laodiceidae. There are, however, no cordyli nor are the small cirrus-like tentacles true marginal cirri.

Until a complete specimen is obtained it seems unnecessary to say more.

I have much pleasure in naming the genus after my friend P. L. Kramp, while giving a specific name which alludes to its uncertain systematic position. I propose the name *Krampella dubia* n.g., n.sp.

The specimen has been deposited in the British Museum (Natural History) and given the registration number B.M. 1957.5.8.1.

My thanks are due to Captain C. A. Hoodless and the crew of R.V. *Sarsia* who collected the specimen; and to Dr J. S. Alexandrowicz who very kindly removed and sectioned one quadrant of the medusa.

REFERENCE

Russell, F. S., 1956. On a new medusa, *Amphinema krampi* n.sp. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 371–3.

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THE LOSS OF MERCURY FROM STORED SEA-WATER SOLUTIONS OF MERCURIC CHLORIDE

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(Text-figs. 1 and 2)

To assess the efficiency of an anti-fouling composition it is necessary to determine the rate at which the poison leaches from the paint film into the surrounding sea water. With a paint containing mercury this estimation is difficult because sea-water solutions of mercuric chloride are unstable. Thus, when examined by the chloroform-dithizone procedure (Harris, 1946) these solutions have been found to lose as much as 80% of their mercury content in the course of a week (Robinson, private communication).

In the present work, ^{203}Hg-labelled mercuric chloride has been used for a quantitative and qualitative study of the loss of mercury from sea water, and the results of this investigation have demonstrated the considerable importance of bacteria as a factor in this process.

One of us (F. H. R.) is indebted to the National Research Council of Canada for a Research Fellowship to work at Plymouth, and the other wishes to acknowledge a Research Fellowship from International Paints Ltd. Both of us wish to express our thanks to Mr O. D. Hunt, Mr T. W. Robinson and Mr R. Robinson for their keen interest in this work, and to Dr H. W. Harvey, F.R.S., for his many helpful suggestions. We should also like to thank Dr B. C. Abbott for his advice on the use of radioactive materials, and Mr L. Hummerstone for the maintenance of the counting equipment.

PRELIMINARY EXPERIMENTS

Chloroform-dithizone titrations

Mercuric chloride was added to filtered Plymouth sea water to give a concentration of 0·1 mg Hg/l. ($0{\cdot}5 \times 10^{-6}$ M), and estimations were made daily by means of the chloroform-dithizone procedure to determine the quantity of mercury present in the solution. The results of experiments with plain sea water, and with sea water to which 40% formaldehyde had been added

(5 ml./l.), are shown in Table 1. For the purposes of tracer isotope studies it was thought necessary to use a higher concentration of mercuric chloride, and further estimations were therefore carried out using a sea-water solution of this compound containing 1 mg Hg/l. The results of this experiment are also included in Table 1. In these experiments, which were carried out at 16–18° C, it was found that after one week only a very small proportion of the mercury originally added to untreated sea water could be detected, but that 80% of the mercury could still be found in sea-water solutions containing formaldehyde.

TABLE 1. RATES OF LOSS OF MERCURY FROM SOLUTIONS OF MERCURIC CHLORIDE IN PLAIN SEA WATER AND IN SEA WATER CONTAINING FORMALDEHYDE

(Concentration of 40% HCHO = 5 ml./l. of sea water. Experiment carried out at 16° C)

	Chloroform-dithizone titres (ml.)		
	Plain sea water		Formaldehyde-treated sea water
Day	(0·1 mg Hg/l.)	(1·0 mg Hg/l.)	(0·1 mg Hg/l.)
0	26	240	25
1	24	230	24
2	20	210	24
3	11	120	24
4	—	50	—
5	—	12	—
6	—	9	—
7	5	—	20

Prevention of mercury loss by various bactericides

It seemed possible that the role of formaldehyde in preventing mercury loss might be chemical rather than bactericidal. Accordingly, estimations were made of the loss of mercury from solutions of mercuric chloride (1 mg Hg/l.) in sea water in which bacterial growth was prevented by several different methods. The results of this experiment are given in Table 2, which shows that, in addition to formalin, other treatments which would destroy bacteria or inhibit their growth, also prevented the loss of mercury from the sea water.

TABLE 2. LOSS OF MERCURY FROM SOLUTIONS OF MERCURIC CHLORIDE IN SEA WATER TREATED TO PREVENT BACTERIAL GROWTH

(Sea water saturated with chloroform and phenol; 40% formaldehyde used at a concentration of 5 ml./l.; penicillin and streptomycin each used at a concentration of 50 mg/l.)

Treatment	Percentage decrease in chloroform-dithizone titration after 1 week
None	74
Chloroform	0
Formaldehyde	0
Phenol	4
Penicillin and streptomycin	9
Autoclaving	5

QUANTITATIVE STUDIES USING TRACER ISOTOPES

To examine whether or not the decrease in the chloroform-dithizone titre represented an actual loss of mercury from the sea water, experiments were carried out with radioactive mercuric chloride.

MATERIALS AND METHODS

Radioactive mercury (^{203}Hg) was obtained from Harwell as mercuric oxide and converted into the chloride by treatment with 50% HCl. Certain technical difficulties encountered in the estimation of radioactive mercury have been described by Kelly, Thorpe, Threefoot & Burch (1950). In the present work it was found that the volatilization of mercury during the drying of radioactive samples was prevented if sodium sulphide was added to the solution before it was warmed to dryness on the planchette. Estimations of radioactivity were usually made with 0·1 ml. samples of the sea-water solution which, when dried, were found to contain about 3 mg solids/cm^2. The correction necessary for self-absorption in these samples was determined by comparing the number of counts/min. shown by samples prepared in sea water and in distilled water. This correction (2%) was then applied in all subsequent estimations of radioactivity in sea-water samples. As a precaution, the decay curve of the radioactive material was plotted from measurements of radioactivity made at suitable intervals and was found to be similar to that shown by ^{203}Hg (half-life = 47·9 days).

ESTIMATION OF MERCURY LOSS

Sufficient radioactive mercuric chloride was added to sea water containing glucose and ferric ammonium citrate (to promote the growth of bacteria) to give a concentration of 1 mg Hg/l. Simultaneous estimations of the mercury present in the sea water by titration with chloroform-dithizone and by measurement of the amount of radioactivity in samples (0·1 ml.) of the sea water were then made immediately after the mercuric chloride was added to the sea water and at suitable intervals during the subsequent week. In addition, simultaneous determinations were made of the quantity of mercury present on bacteria in the solution by passing a sample (10 ml.) through a millipore filter which was then examined for radioactivity.

The results of this experiment (see Fig. 1) showed that during the first 2 days the loss of mercury as determined by chloroform-dithizone titration was approximately the same as that estimated from measurements of radioactivity in the sea water. This correlation disappeared, however, once a significant amount of mercury had been taken up by the bacteria, and as this quantity neared and passed its maximum value the chloroform-dithizone procedure detected much less mercury than was found to be present in the sea water by estimations of radioactivity. It is interesting to note that in this region of the curve the total amount of mercury present in the sea water was roughly the sum of the quantity taken up by bacteria and that detected by the chloroform-dithizone titrations, a finding which implied that the mercury held by the bacteria was not extracted by chloroform-dithizone. Of further interest was the finding that, during the latter part of the experiment, the quantity of mercury held by bacteria in suspension gradually diminished until, after 6 days, it had practically disappeared.

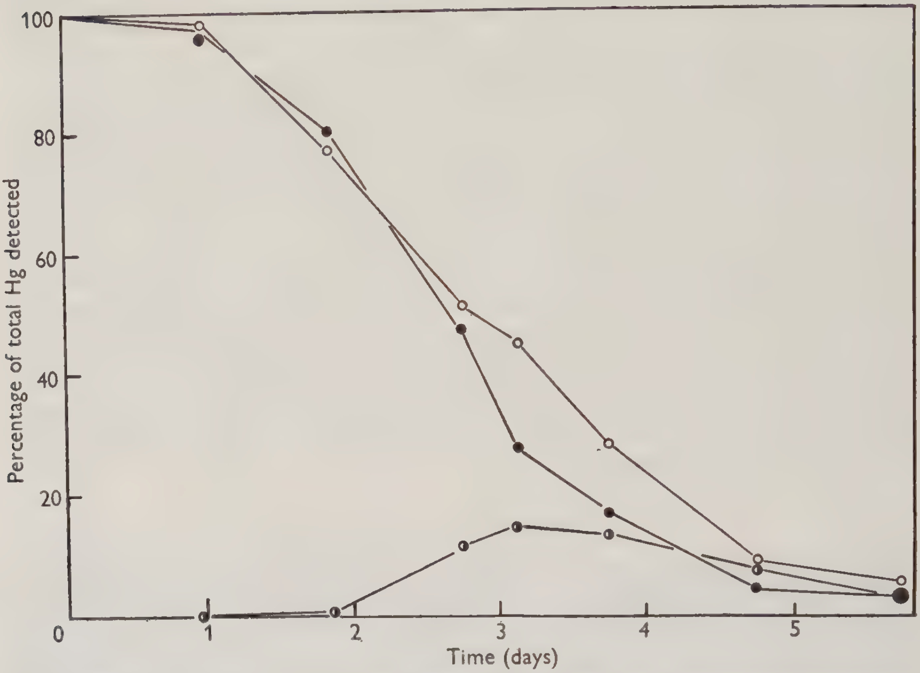

Fig. 1. The loss of mercury as mercuric chloride (1 mg Hg/l.) from sea water as estimated by chloroform-dithizone titrations (●—●), and measurements of radioactivity in solution (○—○). The amounts of mercury present on suspended bacteria are also shown (◑—◑).

LOCATION OF THE LOST MERCURY

Previous experiments had shown that the loss of mercury from stored sea-water solutions of mercuric chloride was genuine, and not simply the results of inadequacies in the chloroform-dithizone procedure. Further experiments were therefore made to locate the mercury which had been lost from solution.

Attachment to walls of storage vessel. In preliminary studies, flasks in which sea-water solutions of mercury had been stored were emptied and fumed out with conc. HNO_3. The acid extract and the cleaned glass were then examined for radioactivity. On no occasion did the mercury so detected account for more than 1 % of that lost. In later experiments, no preliminary treatment with acid was carried out: the flasks were emptied, drained, dried and smashed, and fragments of suitable size were immediately examined for radioactivity. When this procedure was used a much larger amount of mercury was detected, but this was still not enough to account for the total quantity lost. Because of this unexpected finding it became necessary to examine the possibility that some of the mercury had volatilized from solution.

Volatilization from solution. In these experiments a slow stream of air was passed over the sea-water solution and led out to the atmosphere through a series of traps containing various reagents. In the course of these studies the reagents used were ethanol, *o-cyclo*hexanone, distilled-water solutions of cysteine and sodium sulphide, ethyl ether, activated animal charcoal, alumina, powdered silver, acidified permanganate, potassium iodide and sodium hypobromite. However, only charcoal was effective in trapping a significant amount of volatile radioactive compound(s). When the decay curve of this radioactive material trapped on the charcoal was plotted it was found to correspond to that for ^{203}Hg. In different experiments the amounts of mercury detected on the charcoal varied from 5 to 25 % of the quantity lost, the minimum value being obtained in an experiment with sea water containing nutrients, and the maximum with untreated sea water.

These preliminary studies led to three conclusions. The first was that some of the lost mercury had volatilized from solution and some had become attached to the walls of the glass vessel. The second was that the attached mercury could itself be converted into a volatile compound by treatment with conc. HNO_3. Thirdly, it appeared that the distribution of the lost mercury between glass and vapour largely depended upon the conditions under which bacteria developed in the solution. In order to examine these possibilities further, the following experiments were carried out.

INFLUENCE OF NUTRIENTS ON THE PATTERN OF MERCURY LOSS

A sample (200 ml.) of sea water containing 1 mg Hg/l. as ^{203}Hg-labelled mercuric chloride was added to each of two flasks. One of the samples was enriched with glucose (0·5 g/l.) and ferric ammonium citrate (1 mg/l.) to promote the growth of bacteria. No additions were made to the other sample. Each solution was examined daily for distribution of radioactivity between the solution, the suspended bacteria, and the walls of the vessel, the latter quantity being determined by estimating the radioactivity on a glass slide suspended vertically in the liquid.

The results of this experiment (Fig. 2) clearly show the influence of bacteria on mercury loss. Thus, compared with untreated sea water, the enriched sample lost mercury at a faster rate, the bacteria in it took up twice as much mercury as did those in the untreated sample, and about twice the quantity of mercury was lost to a glass slide suspended in the solution. There were, however, certain similarities between the two samples. Thus the amount of mercury removed by suspended bacteria passed through a maximum value at a time when approximately 50 % of the mercury had been lost from solution, and the quantity detected on the glass slide rose to a peak one day later. It seemed surprising that the amount of mercury taken up by the glass slide did not continue to increase throughout the whole experiment, and to check this observation, at the end of the experiment the flasks containing the untreated and the enriched samples were emptied, dried, and smashed, and fragments were examined for mercury in the usual way. When this was done 18 % of the lost

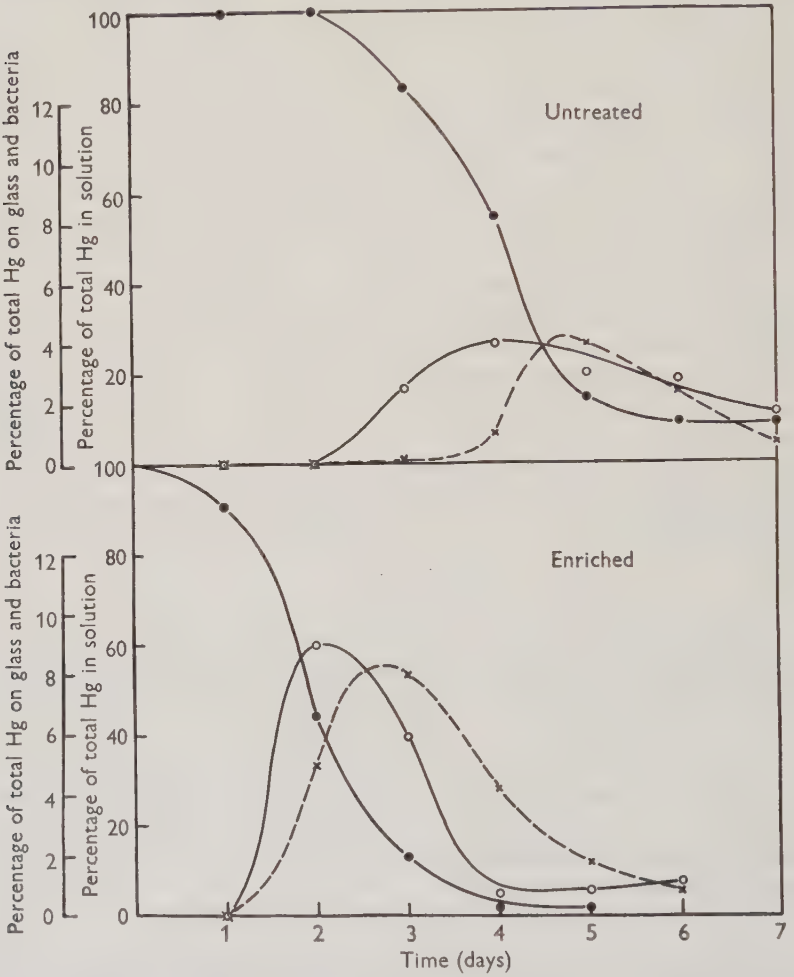

Fig. 2. Distributions of mercury between solution (●—●), suspended bacteria (○—○) and walls of the vessel (×---×) after mercuric chloride (1 mg Hg/l.) had been added to enriched (0·5 g glucose and 1 mg ferric ammonium citrate/l.) and untreated sea water.

mercury was detected on the flask which had contained the enriched sample and 9% on the flask which had held the untreated sample. As the values calculated from the radioactivity of the glass slides accounted for less than 1% of the mercury lost from each sample, it was obvious that during the latter half of the experiment, at least, measurement of the amount of mercury on a suspended glass slide was inadequate as an indication of the quantity adsorbed on the walls of the vessel. The reason for this discrepancy became clear when the distribution of mercury over the flask was examined at the end of the experiment, for whereas very little mercury could be detected on the sides, a large amount was found on the bottom of the vessel. It seemed, therefore, that most of the mercury which was lost from solution to the glass of the storage vessel was not fixed by sessile bacteria but sedimented either attached to bacteria themselves or as some insoluble derivative which they formed. Moreover, this sedimented mercury was very difficult to remove and was probably firmly attached to the bottom of the flask by the slimy bacterial film already present.

Next, an attempt was made to examine the possibility that mercury in a volatile form might be released by lysing the bacteria attached to the walls of the glass vessel. To do this, a sample of enriched sea water containing 1 mg Hg/l. was allowed to stand at room temperature for 6 days, after which the solution was removed from the flask and replaced by acetone. The flask was then sealed, a column of activated animal charcoal was inserted between the acetone and the atmosphere, and a current of air was passed through the system for a further 6 days. The charcoal was then extruded from the column, thoroughly mixed and samples (2–4 mg) then worked into a paste with absolute ethanol on planchettes. The samples were then slowly dried and examined for radioactivity. It was found in this experiment that the quantity of radioactivity on the charcoal column corresponded to 5% of the total amount of mercury originally added to the sea-water sample, and approximately one-third of the mercury attached to the glass of the vessel. An attempt to detect further quantities of volatile mercury by reassembling the apparatus with a column of fresh charcoal and continuing the experiment for a further 4 days was unsuccessful, for no additional quantity of volatile mercury was detected.

Further experiments were carried out in order to examine the influence of nutrients on the distribution of the lost mercury between the atmosphere and the surface of the glass vessel. In these experiments one enriched and one plain sea-water sample containing 1 mg Hg/l. (as ^{203}Hg-labelled mercuric chloride) were placed in each of two flasks. The flasks were then sealed and a current of air was passed over the surface of each solution and out to the atmosphere through a column of activated animal charcoal. In order to avoid dismantling the assemblies, daily estimations of mercury loss from the two solutions were obtained from measurements of the radioactivity in identical samples prepared at the same time but left open to the atmosphere. When these estimations indicated that more than 80% of the mercury originally added to the solutions had been lost, the flasks equipped with charcoal columns were opened and each assembly was examined as follows. The sea water was removed from each flask which was then drained, dried and smashed, and fragments from the sides and bottom of the flask were then examined for radioactivity. Next, the charcoal was extruded from the column and examined for radioactivity. The results of these experiments are shown in Table 3, from which it is clearly seen that whereas a large proportion of the mercury lost from the plain sea-water sample was trapped on the charcoal column, only a small quantity was found on the glass. By contrast, however, in the case of the enriched sample, whereas a large amount of the lost mercury was detected on the glass, a considerably smaller fraction was found on the charcoal. In addition, it was found that the rate of loss of mercury from the plain sea-water solution contained in the flask equipped with a charcoal column was much slower than that from the solution which was exposed to the atmosphere. Thus, although measurements of the radioactivity present in this latter sample indicated that more than 80% of the mercury had gone from the solution in 6 days, similar determinations carried out on the other solution showed that, after 6 days, only 55% of the mercury originally added had been lost.

TABLE 3. INFLUENCE OF NUTRIENTS ADDED TO SEA WATER ON THE PATTERN OF MERCURY LOSS

(Experiment carried out for 6 days at room temperature)

	Enriched sea water (%)	Untreated sea water (%)
Total amount of mercury lost	86	55
Quantity adsorbed on charcoal	15	34
Quantity attached to glass	50	1
Percentage of lost mercury accounted for	76	64

The experiments just described demonstrated that bacteria influence the loss of mercury from sea-water solutions both quantitatively and qualitatively. Thus, sea water enriched with nutrients loses mercury much faster than untreated sea water, and the distribution of the mercury between volatile compounds and the glass surface of the vessel is markedly different in the two cases, for whereas nutrients favour attachment to the glass, absence of nutrients favours volatilization. In conclusion, it should be stated that in none of these experiments has all the lost mercury been detected. However, it has been shown that part of the mercury on bacteria attached to the glass can be released as a volatile compound when these bacteria are lysed, and this finding draws attention to the possibility that some of the mercury may be volatilized from the flasks when these are dried prior to examination for radioactivity.

DISCUSSION

The results of the present study have emphasized the importance of bacteria as a factor determining the loss of mercury from stored sea-water solutions of mercuric chloride. Consequently, when leaching rates of mercury from antifouling compositions are measured, care should be taken to prevent the growth of bacteria in the sea water in contact with the paint film. For not only do bacteria reduce the chloroform-dithizone titres by removing mercury from solution as a volatile compound (or compounds), they also cause a large quantity of the mercury to concentrate at the surface of the glass vessel, in which form it is no longer extracted into chloroform-dithizone.

At present it is not clear why part of the mercury is volatilized from the solution, and part is attached to the walls of the vessel; and, further, why the relative amounts of these fractions are changed by the presence of nutrients in the sea water. However, an observation by Ruska (1947) that certain bacteria can convert mercuric chloride into at least two products may provide a possible answer. For if the bacteria which arise in stored sea water convert the mercury into more than one compound then it is possible that the relative amounts of the compounds produced might depend on the conditions under which the bacteria develop. However, further speculation along these lines is not likely to be profitable until the identities of these compounds have been determined.

SUMMARY

The disappearance of mercury from stored sea-water solutions of mercuric chloride has been studied by means of the chloroform-dithizone procedure and by the use of radioactive mercury (^{203}Hg).

When sea water is enriched with nutrients facilitating the growth of bacteria, the rate at which mercury is lost from the solution is markedly increased. On the other hand, when the sea water is treated in various ways which prevent the development of bacteria, the loss of mercury from solution is considerably reduced.

Rates of loss of mercury measured by the chloroform-dithizone procedure and by the use of tracer isotopes are in good agreement only in the early stages of the experiments. Thus, at a time when approximately half the mercury has been lost, titrations with chloroform-dithizone only account for about 50% of the mercury determined by measurements of radioactivity. Further findings have suggested that this discrepancy arises from the fact that a considerable amount of the mercury is taken up by bacteria and converted into a form which is not detectable with chloroform-dithizone.

A comparison has been made of the patterns of mercury loss from plain sea water and from sea water containing nutrients promoting the growth of bacteria. The mercury lost from the former medium is produced mainly as a volatile compound, only a very small fraction being attached to the glass

surface of the containing vessel. By contrast, a large amount of the mercury lost from the latter medium is attached to the glass and a significantly smaller fraction is volatilized. Some of the mercury attached to bacteria on the walls of the containing vessel can be released in the form of a volatile compound when the bacteria are lysed.

REFERENCES

Harris, J. E., 1946. Report on anti-fouling research. *J. Iron St. Inst.*, Vol. 2, pp. 297–333.

Kelly, F. J., Thorpe, C. R., Threefoot, S. H. & Burch, G. E., 1950. Influence of self-absorption, volatilization and deliquescence in counting of radioelements. *J. Lab. clin. Med.*, Vol. 35, pp. 606–21.

Ruska, H., 1947. Fixation of mercuric chloride on bacteria and virus. *Arch. exp. Path. Pharmak.*, Bd. 204, pp. 576–85.

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THE MODES OF ACTION OF TOXIC AGENTS

II. FACTORS INFLUENCING THE TOXICITIES OF MERCURY COMPOUNDS TO CERTAIN CRUSTACEA

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(Text-figs. 1–3)

It is well known that certain alkylmercuric halides are more toxic than inorganic mercury to a number of widely different test organisms, such as fungi (Yoshiyuki & Shintani, 1942), zooplankton (Hoffman, 1950), bacteria (Okamoto & Nagayama, 1952) and molluscs (Bond & Nolan, 1954). However, although the considerable toxicities of the organomercury compounds have often been observed, their modes of action seem to have attracted little attention.

In a recent investigation (Corner & Sparrow, 1956) the toxicities of ethylmercuric chloride and mercuric chloride were compared using two crustacean species as test animals. It was found that whereas the alkylmercuric compound was far more toxic than mercuric chloride to *Artemia salina* (L.), an animal highly resistant to mercury poisoning, the difference between the toxicities of the two compounds to *Elminius modestus* Darwin, a much less resistant species, was far smaller. These findings led to the view that rates of penetration are important factors influencing the toxicities of mercury compounds, and further evidence in support of this concept has now been obtained from the present work. The relative toxicities of a number of organomercury compounds to *Artemia* and to *Elminius* have been measured, and a study made of the extent to which these values are related to the lipoid solubilities of the compounds and their abilities to interact with proteins.

EXPERIMENTS

GENERAL METHODS

Animals

Artemia salina was reared in the laboratory. Larvae were collected 24 h after they had hatched, concentrated in a small volume of filtered sea water, and samples of the concentrate were added to the various toxic media under test. Adults of *Elminius modestus* were obtained from rocks at Tinside, below the Plymouth Laboratory. They were placed in filtered sea water and the larvae liberated were collected after 1 h and added to the toxic solutions.

Poisons

The organomercury compounds used were the first five members of an homologous series of primary alkylmercuric chlorides and, in addition, *iso*-propyl-, *iso*-amyl- and phenylmercuric chlorides. Inorganic mercury was

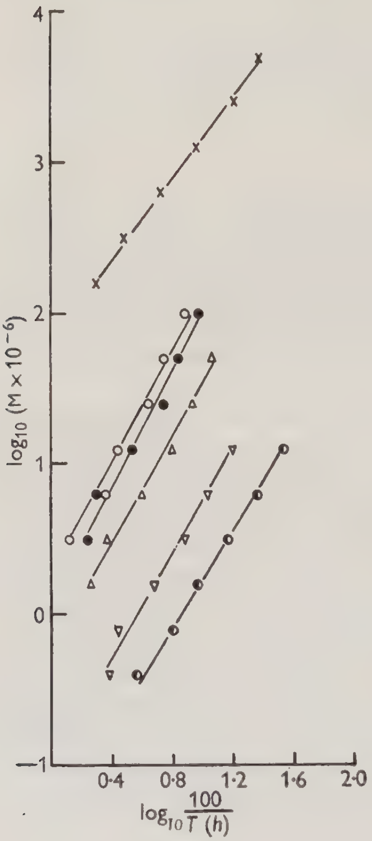

Fig. 1

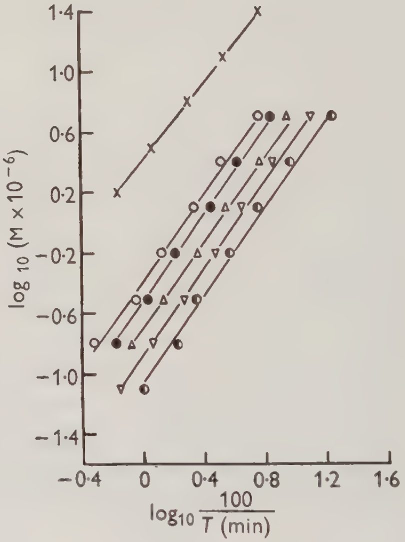

Fig. 2

Fig. 1. Toxicities of mercuric chloride (×—×), methylmercuric chloride (○—○), ethylmercuric chloride (●—●), *n*-propylmercuric chloride (△—△), *n*-butylmercuric chloride (▽—▽) and *n*-amylmercuric chloride (◐—◐) to *Artemia* larvae in sea water, pH 8·1.

Fig. 2. Toxicities of mercuric chloride (×—×), methylmercuric chloride (○—○), ethylmercuric chloride (●—●), *n*-propylmercuric chloride (△—△), *n*-butylmercuric chloride (▽—▽) and *n*-amylmercuric chloride (◐—◐) to *Elminius* larvae in sea water, pH 8·1.

used as mercuric chloride and mercuric iodide. It was found that the organo-mercury compounds were only slightly soluble in sea water, and that as the homologous series was ascended, solubility decreased. In order to obtain a known concentration of each compound in sea water a concentrated solution in warm ethanol (*o-cyclo*hexanone in the case of phenylmercuric chloride) was first prepared, and a measured volume of the solution was then added to the sea water. In this way, sea-water solutions of methyl-, ethyl-, *n*-propyl- and *iso*-propylmercuric chlorides equivalent to 10 mg Hg/l. (5×10^{-5} M) were prepared: the other mercury compounds were used at half this concentration. In all experiments with both species of test animals control tests showed that the quantities of ethanol and *o-cyclo*hexanone present in the toxic solutions had no significant toxic effect.

RELATIVE TOXICITIES

The methods used in toxicity experiments with *Artemia* and *Elminius* were those described in an earlier paper (Corner & Sparrow, 1956). The data from experiments using mercuric chloride and the homologous series of organo-mercury compounds are shown in Fig. 1 (*Artemia*) and Fig. 2 (*Elminius*). By plotting the logarithm of the molar concentration against the logarithm of the reciprocal of the time at which 50% of the animals died (TD_{50}) a linear relationship was found for each poison with each test animal. From these data

TABLE 1. RELATIVE TOXICITIES OF VARIOUS MERCURY COMPOUNDS TO *ARTEMIA*

Standard mercuric chloride solution equivalent to 200 mg Hg/l. (10^{-3} M). TD_{50} values were 12·5 h (Expt. 1) and 16 h (Expt. 2).

	No. of times as toxic as mercuric chloride	
Mercury compound	Expt. 1	Expt. 2
CH_3HgCl	15	15
C_2H_5HgCl	18	17
n-C_3H_7HgCl	47	54
n-C_4H_9HgCl	300	294
n-$C_5H_{11}HgCl$	930	980
i-C_3H_7HgCl	—	25
i-$C_5H_{11}HgCl$	—	450
C_6H_5HgCl	17	23
HgI_2	29	24

TABLE 2. RELATIVE TOXICITIES OF VARIOUS MERCURY COMPOUNDS TO *ELMINIUS*

Standard mercuric chloride solution equivalent to 1 mg Hg/l. (5×10^{-6} M). $TD_{50} = 1$ h.

Mercury compound	No. of times as toxic as mercuric chloride	Mercury compound	No. of times as toxic as mercuric chloride
CH_3HgCl	4·7	i-C_3H_7HgCl	2·4
C_2H_5HgCl	6·8	i-$C_5H_{11}HgCl$	16
n-C_3H_7HgCl	9·7	C_6H_5HgCl	4·3
n-C_4H_9HgCl	16	HgI_2	4·6
n-$C_5H_{11}HgCl$	20		

the concentration of each poison which killed 50% of the test animals in the same time was calculated and compared with that of mercuric chloride as a standard. The ratios of these equitoxic concentrations were then used to determine the relative toxicities of the poisons to each species, and the results are shown in Table 1 (*Artemia*) and Table 2 (*Elminius*). In the course of experiments with *Artemia*, determinations of relative toxicities were made on several occasions over a period of about 6 months, using different batches of test animals at different room temperatures. It was found, however, that the relative toxicities of the poisons, using mercuric chloride as a standard, were fairly consistent (see Table 1).

STUDIES WITH CYSTEINE AND REDUCED GLUTATHIONE

Studies of the nature of heavy metal poisoning have shown that mercury compounds are usually far less toxic to the test material when they are used in the presence of substances which contain a sulphydryl group. Thus, in studies of the inactivation of papain (Hellerman & Perkins, 1934), urease (Hellerman, Chinard & Deitz, 1943; Evert, 1952), succinoxidase (Barron & Kalnitsky, 1947), invertase (Gemmill & Bowman, 1950), amylase and phosphorylase (Ewart, Siminovitch & Briggs, 1953) and yeast carboxylase (Stoppani, Actis, Deferrari & Gonzalez, 1953) it has been found that the inhibitory powers of mercury compounds are much less when the enzyme is protected by such compounds as BAL, cysteine, reduced glutathione and thioglycollic acid. Further, even when the enzyme has been inhibited, the addition of a sulphydryl compound to the system can restore enzymatic activity. Analogous studies carried out with other test material have shown that cysteine and reduced glutathione counteract the inhibitory effects of mercury compounds on the growth of bacteria (Jude, Nordmann, Girard, Nordmann, Servant & Gauchery, 1952) and partially prevent the toxic effects of mercury and copper on *Daphnia magna* (see Holm-Jensen, 1948).

Because of these findings, it seemed of interest to examine the possibility that the toxic effects of some of the mercury compounds used in the present work might be diminished when cysteine or reduced glutathione was added to the toxic solutions: further, that after the test animals had been poisoned, subsequent treatment with thiol compounds might facilitate their recovery. The following experiments were, therefore, carried out.

Protection studies

Using *Elminius* as the test animal, each of six mercury compounds was examined for toxicity when used alone, and when used with a tenfold excess of reduced glutathione. It was found that the thiol compound completely abolished the toxicity of mercuric chloride, and considerably reduced those of methyl-, ethyl- and *n*-propylmercuric chlorides. By comparison, however, its influence on the toxicity of *n*-butylmercuric chloride was much less, and still less with *n*-amylmercuric chloride (see Table 3).

When a similar study was made with *Artemia*, mercuric chloride could not be used because of the large amounts of glutathione needed to provide a tenfold excess of a lethal concentration of the poison. The results of experiments with the other mercury compounds, however, showed that the toxicities of methyl-, ethyl-, *n*-propyl- and *n*-butylmercuric chlorides were considerably reduced by both cysteine and reduced glutathione, the latter compound being the more effective of the two. Moreover, as in the experiments with *Elminius*, it was found that the protection which either thiol compound provided against poisoning by *n*-amylmercuric chloride was noticeably less than that observed when any of the other four mercury compounds was used (see Table 4).

TABLE 3. THE EFFECT OF REDUCED GLUTATHIONE ON DEATH-RATES OF *ELMINIUS* IN SOLUTIONS OF VARIOUS MERCURY COMPOUNDS

Each poison used at a concentration giving a TD_{50} of 1 h. Reduced glutathione used in tenfold excess of each mercury compound.

Mercury compound	Percentage increase in TD_{50}	Mercury compound	Percentage increase in TD_{50}
$HgCl_2$	∞*	n-C_3H_7HgCl	800
CH_3HgCl	900	n-C_4H_9HgCl	420
C_2H_5HgCl	870	n-$C_5H_{11}HgCl$	410

* Animals died no faster than controls.

TABLE 4. EFFECTS OF CYSTEINE AND REDUCED GLUTATHIONE ON THE TOXICITIES OF MERCURY COMPOUNDS TO *ARTEMIA*

Each poison used in a concentration giving TD_{50} of 10 h. Thiol compounds used in tenfold excess of each poison.

	Percentage increase in TD_{50}	
Mercury compound	Cysteine	Reduced glutathione
CH_3HgCl	84	240
C_2H_5HgCl	87	260
n-C_3H_7HgCl	89	280
n-C_4H_9HgCl	92	270
n-$C_5H_{11}HgCl$	20	96

Recovery studies

Elminius larvae were placed in sea-water solutions of mercuric chloride (1 mg Hg/l.) and *n*-amylmercuric chloride (0·05 mg Hg/l.) for measured times. The toxic solutions were then centrifuged, the supernatants were decanted off and the larvae were suspended in fresh sea water. After a second centrifuging, half the animals were transferred to plain sea water and the remainder to a sea-water solution of reduced glutathione (5×10^{-5} M). The rates of death of the two sets of animals were then compared. It was found that animals which had 'died' (i.e. become completely quiescent) in the toxic media did not recover after they had been transferred either to plain sea water or to sea water containing glutathione. It was also observed that, of the animals which had lost their ability to move freely in the toxic solutions, a few completely recovered their mobility when they were transferred to the new media, and the remainder continued to die at a very slow rate. However, of the latter animals, those placed in sea water containing glutathione died much more slowly than the larvae transferred to plain sea water (Table 5).

TABLE 5. EFFECT OF REDUCED GLUTATHIONE ON *ELMINIUS* PREVIOUSLY TREATED WITH MERCURY

GSH = reduced glutathione (5×10^{-5} M) in sea water (SW).

Mercury compound	Time of immersion (min)	Second medium	TD_{50} in second medium (h)
$HgCl_2$	15	SW	29
	15	GSH	42
	30	SW	14
	30	GSH	21
$n\text{-}C_5H_{11}HgCl$	15	SW	17
	15	GSH	26
	30	SW	9
	30	GSH	21

TABLE 6. EFFECT OF REDUCED GLUTATHIONE ON *ARTEMIA* PREVIOUSLY TREATED WITH MERCURY

GSH = reduced glutathione (5×10^{-4} M) in sea water (SW).

Mercury compound	Time of immersion (h)	Second medium	TD_{50} in second medium (h)
$HgCl_2$	4	SW	108
		GSH	115
$n\text{-}C_5H_{11}HgCl$	4	SW	45
		GSH	57

An experiment identical with the one just described was carried out with *Artemia*. It was found that the influence of reduced glutathione on the death-rate of the animals was negligible (see Table 6).

INHIBITION OF UREASE BY VARIOUS MERCURY COMPOUNDS

Mention was made earlier of studies of the effects of heavy metals on enzymes. These investigations were primarily concerned with establishing whether or not a certain enzyme possessed sulphydryl groups, and the extent to which such groups influenced catalytic activity. More recently, however, Okamoto & Nagayama (1952) have compared the relative abilities of a number of mercury compounds as inhibitors of catalase and as bactericides in order to test the possibility that there was a correlation between their abilities to react with proteins and their potencies as antibacterial agents. No correlation was found. In the present work, similar experiments were carried out to see whether or not the affinities of mercury compounds for protein, estimated from their abilities to inhibit urease, might be correlated with the toxicities of these compounds to *Artemia* and *Elminius*.

Because the purpose of this study was simply to compare the toxicities of a number of substances to urease, it was not thought necessary to prepare the enzyme in pure crystalline form. Commercial preparations, however, were found to be much too inactive and, accordingly, the enzyme was obtained by aqueous-acetone extraction of Jack-bean meal in the manner described by Sumner (1926). This procedure usually gave a preparation with an activity of 0·20 Sumner units/mg. Various concentrations of each mercury compound in ethanol were added to solutions (5 ml.) of the enzyme (10 Sumner units) in 0·01 M phosphate buffer, pH 6·7 (the amounts of ethanol used had no influence on enzyme activity). Each mixture was allowed to stand for 15 min at room temperature and its ureolytic activity was then compared with that of a control solution, using the colorimetric method of Van Slyke & Archibald (1944). In this way, the extent of the inactivation caused by each concentration of each mercury compound was measured, and the quantity of each poison required to cause a 50% loss of ureolytic activity was estimated. The potencies of the various compounds studied as inhibitors of urease, expressed in terms of that of mercuric chloride as a standard, are shown in Table 7.

TABLE 7. RELATIVE POTENCIES OF VARIOUS MERCURY COMPOUNDS AS INACTIVATORS OF UREASE

Mercury compound	No. of times as effective as mercuric chloride	Mercury compound	No. of times as effective as mercuric chloride
CH_3HgCl	0·76	i-C_3H_7HgCl	0·63
C_2H_5HgCl	0·66	i-$C_5H_{11}HgCl$	0·95
n-C_3H_7HgCl	0·70	C_6H_5HgCl	0·95
n-C_4H_9HgCl	0·76	HgI_2	1·09
n-$C_5H_{11}HgCl$	0·88		

ETHER:SEA-WATER PARTITION COEFFICIENTS OF MERCURY COMPOUNDS

From a study of the permeability of rabbit skin to ethyl-iodide, methanol, ethanol, thiourea, glycerol, urea and glucose, Treherne (1956) found that the rates of penetration of these compounds were closely related to the corresponding ether:water partition coefficients. This result provided quantitative demonstration of an earlier view put forward by Calvery, Draize & Laug (1946) and by Rothman (1943) that the lipoid solubility of the penetrating substance was an important factor in the mechanism of skin permeability.

Because of the possibility that penetration of a lipoid barrier might be an important factor influencing the toxic actions of mercury compounds, it was considered worth while to examine whether or not a close parallel could be found between the ethyl ether:sea-water partition coefficients of these poisons and their toxicities. Accordingly, the following experiments were carried out using *Elminius* as the test animal.

Each mercury compound was dissolved in sea water to give a concentration corresponding to 1 mg Hg/l. (5×10^{-6} M). A volume of this solution (50 ml.) was then shaken with an equal volume of ethyl ether for 1 min in a separating funnel. After the two phases had separated the lower one was run off and a stream of air was passed through it for 20 min to remove the ether. The quantity of the mercury compound left in this ether-free solution was then determined by comparing its toxicity to *Elminius* with that of a series of sea-water solutions containing known amounts of the mercury compound. The quantity so determined, when subtracted from the amount originally added to the sea water (1 mg Hg/l.), gave the amount extracted by the ether. The ratio of this latter quantity to that remaining in the sea water was then estimated as the partition coefficient.

Because the toxicity data for the mercury compounds had been expressed in terms of mercuric chloride as a standard poison, it was thought necessary to express their lipoid solubilities in a similar way. For this reason it was important that an accurate value should be obtained for the ethyl ether: sea-water partition coefficient of mercuric chloride. It was found, however, that the accuracy of the bio-assay method proved inadequate in the case of mercuric chloride because only a relatively small quantity of this compound was extracted into the ether phase. Accordingly, the value for mercuric chloride was obtained from the use of tracer isotopes. As details concerning the use of radioactive mercury are given elsewhere (Corner & Rigler, 1957), only a brief description of the experimental procedure is included in the present account. ^{203}Hg-labelled mercuric chloride was added to sea water to give a concentration equivalent to 1 mg/l., and estimates of radioactivity in samples (0·1 ml.) were immediately made. After the sea water (50 ml.) had been extracted with an equal volume of ether for 1 min it was again examined for radioactivity in order to determine the quantity of mercury which had been extracted by the ether. From these data the partition coefficient was estimated in the usual way and was found to be 0·26.

Further experiments were carried out with ^{203}Hg-labelled mercuric chloride and *n*-amylmercuric chloride in order to examine the possibility that the aeration of sea-water solutions of mercury compounds in order to remove traces of ether might cause volatilization of the compounds and lead to further errors in the bio-assay method. In these experiments, small quantities of ether were added to sea-water solutions of the two mercury compounds (each equivalent to 1 mg ^{203}Hg/l.) and the media were aerated until the amounts of ether in them were sufficiently small to have no influence on the viability of the test animals (20 min). Determinations of radioactivity in these solutions after aeration had taken place showed that no loss of mercury had occurred and this procedure, therefore, introduced no errors into the bio-assay method.

The partition coefficient for each mercury compound, expressed in terms of that for mercuric chloride as a standard, is shown in Table 8.

TABLE 8. RELATIVE VALUES OF ETHYL ETHER:SEA-WATER PARTITION COEFFICIENTS OF VARIOUS MERCURY COMPOUNDS

Experimental conditions as given in text. Value for standard $HgCl_2$ solution (5×10^{-6} M) = 0·26 at 15° C.

Mercury compound	No. of times partition coefficient greater than that of mercuric chloride	Mercury compound	No. of times partition coefficient greater than that of mercuric chloride
CH_3HgCl	15·9	*i*-C_3H_7HgCl	42
C_2H_5HgCl	63	*i*-$C_5H_{11}HgCl$	1641
n-C_3H_7HgCl	255	C_6H_5HgCl	360
n-C_4H_9HgCl	879	HgI_2	18·9
n-$C_5H_{11}HgCl$	2490		

RESULTS

Ferguson (1939) has drawn attention to the fact that the logarithms of equitoxic concentrations of members of an homologous series decrease linearly as the series is ascended, and that a similar relationship holds when certain physical constants (e.g. water solubility, vapour pressure, etc.) of these compounds are expressed in the same way. In the present work it has been found that if the toxicity data for ethyl-, *n*-propyl-, *n*-butyl- and *n*-amylmercuric chlorides are plotted in the manner shown in Fig. 3, linear relationships exist when the compounds are tested with either *Artemia* or *Elminius*. Differences between toxicities to *Artemia*, however, are far greater than those observed when *Elminius*, which is much more readily poisoned, is used as the test species. These observations confirm and extend the results of some earlier experiments (Corner & Sparrow, 1956) which have shown that orders of difference between the toxicities of certain mercury compounds to a test animal are much greater when the animal used is one which is highly resistant to mercury poisoning. It will also be seen from Fig. 3 that stepwise increments in toxicity to *Artemia*, but not to *Elminius*, approximate to corresponding increments in lipoid solubility, consistent with the view that toxicity to *Artemia* is much more influenced by lipoid solubility than is toxicity to *Elminius*.

Two secondary alkylmercuric halides have been studied in the present work, and these poisons have been found to be significantly less toxic than the corresponding primary compounds to both the test species. Nevertheless, as with the primary compounds, the lipoid solubilities of *iso*-propyl- and *iso*-amylmercuric chlorides are more closely related to their toxicities to *Artemia* than to their toxicities to *Elminius*. Similar results have also been obtained in experiments with methylmercuric chloride and mercuric iodide. An exception, however, is phenylmercuric chloride, which possesses a much greater lipoid solubility than might be expected from its toxicity to either species.

It has been found that thiol compounds protect both *Elminius* and *Artemia* from poisoning by mercuric chloride and primary alkylmercuric chlorides. However, further experiments have shown that when the lipoid solubility of the poison is very high (e.g. *n*-amylmercuric chloride) thiol compounds provide less protection than when the poison used is one of low lipoid solubility (e.g. mercuric chloride). Interest also attaches to the additional finding that when lipoid solubilities greatly influence the toxicities of organomercury compounds (experiments with *Artemia*) protection of the test animal by thiols is much smaller than under conditions where lipoid solubilities are of less importance (experiments with *Elminius*). Further, when in complementary studies the test animals have been given preliminary treatments with mercury compounds and measurements subsequently made of their rates of death in plain sea water, it has been found that adding reduced glutathione to the sea water has little effect on the viability of *Artemia*, but a marked influence on

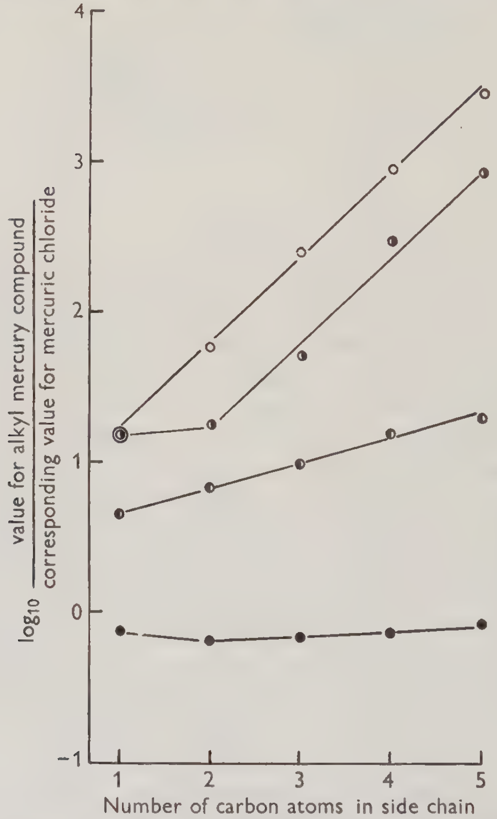

Fig. 3. Toxicity and other data for primary alkylmercuric chlorides. Lipoid solubility, ○—○; toxicity to *Artemia*, ◑—◑ ; toxicity to *Elminius*, ◐—◐ ; potency as urease inhibitor, ●—●.

that of *Elminius*. A possible explanation of this finding is that the ability of a given poison to interact with proteins on the surface of the test animal may play a more important part in determining its toxicity to *Elminius* than its toxicity to *Artemia*. A further possibility is that reduced glutathione may be able to penetrate *Elminius* but not *Artemia* and inactivate some of the mercury poison after it has reached tissues inside the test animal.

Finally, it is obvious from Fig. 3 that differences between the toxicities of mercury compounds to either *Artemia* or to *Elminius* are significantly greater than corresponding differences between the potencies of the poisons as inhibitors of urease, a finding which lends further support to the view that, in general, the toxicities of these compounds are influenced by their lipoid solubilities far more than by their abilities to combine with proteins.

DISCUSSION

Of the possible mechanisms of action of organomercury poisons, one is that the compounds may interact with the surface of the test animal, and another is that these poisons may penetrate into the tissues and inhibit metabolism. If the poisons exerted their toxic action solely by acting on the surface of the animal it might be expected that a reasonable correlation would be found between the toxicities of the poisons to the test animal and their abilities to combine with proteins. However, neither in the present work, nor in studies made by other workers (Okamoto & Nagayama, 1952) has any correlation been found. Moreover, if surface effects alone are responsible for the toxicities of organomercury compounds it would seem unlikely that great differences would be found between the resistances of different test species to the poisons. In this, and a previous study (Corner & Sparrow, 1956), however, the resistance of *Artemia* to mercury poisons has been found to be of a much higher order than that of *Elminius*.

If, however, in order to penetrate the test animal organomercury compounds had to pass through a lipoid barrier, it would be expected that the toxicities of these compounds would largely depend on their lipoid solubilities: and, further, that the importance of lipoid solubility in determining toxicity would vary with different animals according to whether or not a lipoid barrier was dominant among the factors involved in resistance. In general, the findings made in the present study have shown that the toxicities of organomercury compounds are, in fact, related to the lipoid solubilities of these poisons: and, in addition, that whereas this correlation is close in the case of *Artemia*, it is less so in experiments with *Elminius*. These findings are, therefore, consistent with the view that the mechanism of action of an organomercury compound involves penetration of the test animal.

It is necessary, however, to consider the possibility that in order to act on the animal, the poison may not have to penetrate into the tissues and inhibit metabolic changes, but may exert its toxic effects by some physical mechanism as soon as it has entered the lipoid barrier. Although the results of the present work provide no definite information on this point it is possible that the anomalous findings made with phenylmercuric chloride are best explained by assuming that interference with metabolism in the tissues of the animal does, in fact, occur. Thus, if phenylmercuric chloride exerted its toxic action once it had entered the lipoid barrier, it is difficult to see why the toxicity of this poison should be so much smaller than one might expect from its considerable lipoid solubility. On the other hand, if this compound became involved in metabolic processes, then it would be possible to account for its reduced toxicity by assuming that it underwent changes in the tissues which resulted in its detoxication. Although mechanisms of detoxication in *Artemia* and *Elminius* are completely unknown, it is possible that these animals may be able to carry out some of the reactions whereby other species are known to detoxicate administered organic compounds (cf. Young, 1939; Williams, 1949). Thus, it is possible that phenylmercuric chloride, like certain other aromatic compounds, might be conjugated *in vivo* with cysteine and so rendered less toxic. In addition, hydroxylation of the aromatic ring might occur, followed by conjugation of the product so formed with sulphuric or glucuronic acids.

Many of the conclusions drawn from the results of the present study are based on the assumption that *Artemia* is able to survive in the presence of large concentrations of mercury compounds simply because these poisons penetrate the species very slowly. It is possible, however, that mercury compounds may enter *Artemia* at rates commensurate with those at which they penetrate *Elminius*, a species which is much more readily poisoned, and that *Artemia* is highly resistant to the poisons either because it possesses a very efficient means of detoxicating them, or because it can tolerate far higher concentrations of the poisons in its tissues. In order to examine these possibilities further, a study is at present being made in which ^{203}Hg-labelled mercuric and *n*-amylmercuric chlorides are being used to measure the rates of uptake of these compounds by *Artemia* and *Elminius*.

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SUMMARY

A study has been made of the toxicities of mercuric chloride, mercuric iodide and methyl-, ethyl-, *n*-propyl-, *n*-butyl-, *n*-amyl-, *iso*-propyl-, *iso*-amyl- and phenylmercuric chlorides to larvae of the crustaceans *Artemia salina* and *Elminius modestus*. With both species it has been found that all the mercury compounds are more toxic than mercuric chloride, that primary alkylmercuric chlorides are more toxic than the corresponding secondary compounds, and that as the homologous series of primary compounds is ascended, toxicities increase. In addition, it has been found that *Elminius* is much more readily poisoned than *Artemia* by each mercury compound, and that differences between the toxicities of the poisons to *Elminius* are much smaller than corresponding differences observed in experiments with *Artemia*.

It has been found that the toxicities of primary alkylmercuric chlorides to both species are considerably less when cysteine and reduced glutathione are added to the toxic media. However, these thiol compounds give less protection against poisoning by the higher homologues. Other experiments have shown that when the animals are given a preliminary immersion in toxic solutions of mercuric chloride and *n*-amylmercuric chloride, and are then transferred to fresh sea water containing reduced glutathione, the influence of the thiol compound on the subsequent death-rate of *Elminius* is significant, but negligible in the case of *Artemia*.

Measurements have been made of the potencies of the compounds as inhibitors of urease in order to estimate their abilities to combine with proteins. No correlation has been found between these abilities and the toxicities of the compounds to either test species.

The lipoid solubilities of the compounds have been estimated from measurements of their ethyl ether: sea-water partition coefficients. In general, a good measure of agreement has been found between the relative lipoid solubilities of both primary and secondary alkylmercuric chlorides and their respective toxicities to *Artemia*. In addition, it has been found that although this correlation is not so exact for *Elminius*, nevertheless, compounds with high lipoid solubilities have usually been found to be more toxic. However, phenylmercuric chloride is an exception in that its lipoid solubility is much higher than would be expected from its toxicity to either test species.

The results of this study, which in general are consistent with the view that organomercury compounds act by penetrating the test animals, have been discussed.

REFERENCES

Barron, E. S. & Kalnitsky, G., 1947. The inhibition of succinoxidase by heavy metals and its reactivation by dithiols. *Biochem. J.*, Vol. 41, pp. 346–51.

Bond, H. W. & Nolan, N. O., 1954. Results of laboratory screening tests of chemical compounds for molluscicidal activity. II. Compounds of mercury. *Amer. J. trop. Med. & Hyg.*, Vol. 3, pp. 187–90.

CALVERY, H. O., DRAIZE, J. H. & LAUG, E. P., 1946. The metabolism and permeability of normal skin. *Physiol. Rev.*, Vol. 26, pp. 495–540.

CORNER, E. D. S. & RIGLER, F. H., 1957. The loss of mercury from stored sea-water solutions of mercuric chloride. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 449–58.

CORNER, E. D. S. & SPARROW, B. W., 1956. The modes of action of toxic agents. I. Observations on the poisoning of certain crustaceans by copper and mercury. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 531–48.

EVERT, H. E., 1952. Reversibility of mercuric chloride inhibitions of urease. *Fed. Proc.*, Vol. 11, p. 209.

EWART, M. H., SIMINOVITCH, D. & BRIGGS, D. R., 1953. Studies on the chemistry of the living bark of the black locust tree in relation to frost hardiness. VI. Amylase and phosphorylase systems of the bark tissues. *Plant Physiol.*, Vol. 28, pp. 629–44.

FERGUSON, J., 1939. The use of chemical potentials as indices of toxicity. *Proc. Roy. Soc. Lond.* B, Vol. 127, pp. 387–404.

GEMMILL, C. L. & BOWMAN, E. M., 1950. Effects of mercurial compounds on invertase. *J. Pharmacol.*, Vol. 100, pp. 244–9.

HELLERMAN, L., CHINARD, P. C. & DEITZ, V. R., 1943. Protein sulphydryl groups and the reversible inactivation of the enzyme urease. *J. biol. Chem.*, Vol. 107, pp. 443–62.

HELLERMAN, L. & PERKINS, M. E., 1934. Papain activity as influenced by oxidation-reduction and by the action of metal compounds. *J. biol. Chem.*, Vol. 107, pp. 241–55.

HOFFMAN, C., 1950. Beiträge zur Kenntnis der Wirkung von Giften auf marine Organismen. *Kieler Meeresforsch.*, Bd. 7, pp. 443–62.

HOLM-JENSEN, I., 1948. Osmotic regulation in *Daphnia magna* under physiological conditions and in the presence of heavy metals. *Biol. Medd. Kbh.*, Bd. 20, No. 11, 64 pp.

JUDE, A., NORDMANN, J., GIRARD, P., NORDMANN, R., SERVANT, P. & GAUCHERY, O., 1952. Action of certain inhibitors on bacterial enzymic activity. III. Bacterial study of mercurial inhibitors. *Rev. Immunol.*, Vol. 16, pp. 286–99.

OKAMOTO, G. & NAGAYAMA, M., 1952. Physiochemical properties of aqueous solutions of mercury compounds. *Jap. J. Pharm. & Chem.*, Vol. 24, pp. 358–62.

ROTHMAN, S., 1943. The principles of percutaneous absorption. *J. Lab. clin. Med.*, Vol. 28, pp. 1305–21.

STOPPANI, A. O. M., ACTIS, A. S., DEFERRARI, J. O. & GONZALEZ, E. L., 1953. The role of sulphydryl groups of yeast carboxylase. *Biochem. J.*, Vol. 54, pp. 378–90.

SUMNER, J. B., 1926. The isolation and crystallisation of the enzyme urease. *J. biol. Chem.*, Vol. 69, pp. 435–41.

TREHERNE, J. E., 1956. The permeability of skin to some non-electrolytes. *J. Physiol.*, Vol. 133, pp. 171–80.

VAN SLYKE, D. D. & ARCHIBALD, R. M., 1944. Manometric, titrimetric and colorimetric methods for measurement of urease activity. *J. biol. Chem.*, Vol. 154, pp. 623–42.

WILLIAMS, R. T., 1949. *Detoxication Mechanisms*, 288 pp. London: Chapman and Hall.

YOSHIYUKI, I. & SHINTANI, G., 1942. Seed disinfectants. II. Lethal effect of alkylmercuric halides on micro-organisms. *J. agric. Chem. Soc. Japan*, Vol. 18, pp. 1145–48.

YOUNG, L., 1939. The detoxication of carbocyclic compounds. *Physiol. Rev.*, Vol. 19, pp. 323–52.

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THE AGE AND GROWTH OF THE SCALLOP, *PECTEN MAXIMUS* (L.), IN MANX WATERS

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(Text-figs. 1–9)

MATERIAL AND METHODS

An important fishery for the scallop, *Pecten maximus* (L.), is carried on during the winter months round the Isle of Man. A knowledge of the age and growth of the scallop would be useful should conservation become necessary with regard to the fishery.

Regular samples of scallops were obtained at roughly weekly or fortnightly intervals throughout the period October 1950–October 1952 from two areas off Port Erin. These were one-quarter to one-half a mile off Bay Fine (Station 1) and one-quarter to one-half a mile off Bradda Head (Station 2) respectively, the depth in each area being 13–16 fm. (23·8–29·3 m) (see chart, Fig. 1). A few samples were obtained from each of three other stations, in 9–11 fm. (16·5–20·1 m) off Gob-yn-Ushtey (Station 3), in 19–20 fm. (34·7–36·6 m) on the Breast (Station 4), and in 28–29 fm. (51·2–53·0 m) $3\frac{1}{2}$ miles W.S.W. of the Chicken Rock (Station 5).

Excepting a few from commercial fishermen, all samples were taken by the research vessels *William Herdman*, *Cypris* and *Runa* of the Marine Biological Station, Port Erin, towing toothed scallop dredges. The bag of the commercial dredge used by the 64 ft. R.V. *William Herdman* had a belly of rings of $\frac{1}{4}$ in. (6·4 mm) steel, with an internal diameter of $3\frac{1}{4}$ in. (82 mm), joined together by small steel ties, and a back of sisal netting measuring 2–3 in. (51–76 mm) from knot to knot. The cross-bar was 6 ft. (1·83 m) long and carried teeth, $3\frac{1}{2}$ in. (89 mm) apart, which protruded $1\frac{3}{4}$ in. (44 mm). Such a dredge captures only a few small scallops, and it was assumed that the size of the rings and mesh of the bag were the effective factors in selection. A smaller dredge was fitted with an inner lining of $\frac{3}{8}$ in. (9·5 mm) mesh shrimp netting in an attempt to catch smaller members of the scallop population. This small-mesh dredge had a cross-bar 4 ft. (1·22 m) long, carrying teeth 3 in. (76 mm) apart which protruded 2 in. (51 mm). The *Cypris* and *Runa*, which are considerably smaller than the *William Herdman*, used 4 ft. (1·22 m) and 3 ft. (0·91 m) dredges, both commercial and small-mesh. Baird & Gibson (1956) have since suggested that tooth-spacing is the effective agent in size selection, but the small-mesh dredge, nevertheless, took numbers of small scallops by digging deep into the bottom and filling up with gravel and shells, thus acting as a complete bottom sampler.

Individual hauls varied in duration from 7 to 20 min. and in speed of towing from 2 knots (by the *Cypris* and *Runa*) to 3 knots (by the *William Herdman*).

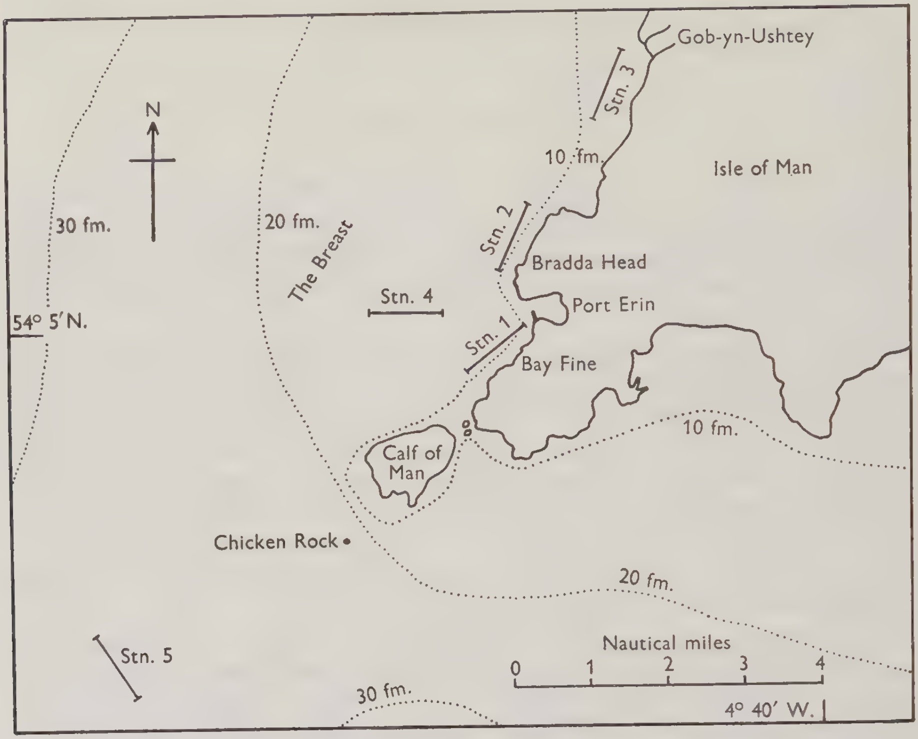
Gob-yn-Ushtey
Stn. 3
N
10 fm.
Isle of Man
30 fm.
20 fm.
The Breast
Stn. 2
Bradda Head
Stn. 4
Port Erin
54° 5′ N.
Stn. 1
Bay Fine
10 fm.
Calf of Man
Chicken Rock
20 fm.
Nautical miles
0
1
2
3
4
Stn. 5
30 fm.
4° 40′ W.

Fig. 1. Chart of S.W. Isle of Man, showing the sampling stations.
Depth contours in fathoms.

Various measurements were made on the scallops caught. The over-all length (anterior-posterior axis), breadth (dorso-ventral axis), and thickness (lateral axis) were measured on a specially designed measuring board. The over-all length and breadth are in reality the length and breadth of the rounded, or right, valve of the shell, which overlaps the flat, or left, valve. Dividers were used to measure the distance from the umbo to the edge of the flat valve, and to each of the annual growth rings (see below) on the flat valve along the dorso-ventral axis. All measurements were to the nearest millimetre below the value shown on the scale.

AGE-ANALYSIS

The growth-rings

The shell of *Pecten maximus* bears distinct concentric growth-rings, which are white in colour and translucent. They show more clearly on the reddish brown flat valve than on the white round valve. They occur regularly and in approximately the same position on most shells.

In addition, the shell bears numerous regularly occurring concentric striae, 0·1–0·3 mm apart, which are prominent and raised over most of the shell, but less so in a slightly concave area within 20–25 mm of the umbo. These striae are the result of the manner in which the shell is enlarged by the deposition of new material at its edge (Coker, Shira, Clark & Howard, 1921), so that each one represents the edge of the shell at the time of its deposition. The striae tend to become worn on the round valve of the shell, which is in contact with the sea-bed, and, on account of this, they were examined on the upper, flat valve.

The growth-rings are ½ mm or less wide, and are made up of striae which are crowded together about 0·05 mm apart and less prominent than elsewhere. Immediately outside a ring, on the side away from the umbo, the striae become more raised, assume a pale brown colour, and become farther (about 0·3 mm) apart. After a few millimetres the striae take on a darker hue, and become gradually closer together until, just before the next ring, they are about 0·1 mm apart. This description applies to the shell between the second and third rings from the umbo. Farther from the umbo, both the growth rings and the striae are somewhat closer together, but a similar series of changes is seen.

Dakin (1909) considered that the growth-rings may indicate the age of the shell. Priol (1930) and Elmhirst (1945) also considered that they were laid down annually. Tang (1941), working on *P. maximus* at Port Erin, found that a ring is laid down in April, May or June, and said that, while it is not certain that all rings are laid down at yearly intervals, there is probably a good deal of agreement between the number of rings and the age of the scallop. I examined the edge of the flat valve of the shell throughout the year, and found that only one ring is laid down each year, in the spring.

The growth-ring is narrow and is laid down slowly, taking perhaps several weeks, but its deposition is followed at once by rapid growth, so that any growth-ring near the edge of the shell has very recently been laid down. In each month the number of scallops was noted which had a ring within 3 mm of the edge of the flat valve. Growth becomes slower in older scallops, and the later growth-rings become close together and difficult to distinguish, and so only scallops which had fewer than five growth-rings were included. The results are shown in Fig. 2. The somewhat low percentages are due to the long period of time (March–May) during which rings are laid down, so that some scallops have acquired more than 3 mm of new growth before others have commenced to lay down their ring.

Evidence that only one ring was laid down each year was afforded also by a tagging experiment. Six living tagged scallops were recaptured which had spent one spring in the sea, and each of these had one more ring than when it was released. Of thirty-seven scallops recaptured before they had spent a spring in the sea, none had acquired a further ring. Occasionally a disturbance-ring was found on these scallops where the edge of the shell was at the time of tagging, being probably caused by a retraction of the mantle edge away from the edge of the shell during tagging. Such a ring differs from an annual ring in that it has no small, crowded striae, and that the striae on either side of it are equally spaced. Such false rings also occur occasionally in nature.

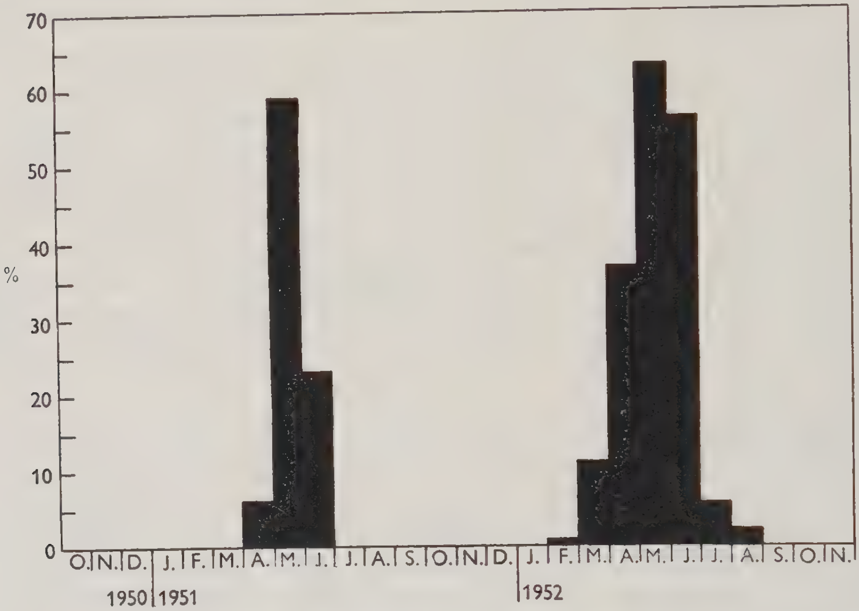

Fig. 2. Percentages of scallops with fewer than five growth-rings, which had a growth-ring within 3 mm of the edge of the shell (3592 scallops examined).

Gibson (1956), by means of a similar tagging experiment, obtained evidence of the annual nature of the growth-rings on the shell of *P. maximus* in Irish waters.

In subsequent pages, the area of shell between two successive annual growth-rings, or between the umbo and the first ring, will be called a growth-band. A scallop is aged by the number of growth-rings and the presence or absence of new growth outside the outermost ring. Thus 5+ indicates that the shell has five rings, with new growth outside the fifth, while 4 indicates that the fourth ring has just been laid down at the edge of the shell.

Length of life

Tang (1941) recorded a scallop, captured off Port Erin, which had twenty-two growth-rings; the oldest one I caught had eighteen rings.

GROWTH

THE FIRST GROWTH-BAND

If the size-frequency of the first growth-band is plotted, a bimodal distribution is obtained; Bradda and Bay Fine scallops with from 1 to 15 rings were used for this investigation. The results are presented in Fig. 3, the widths being in 2 mm groups. The modal values are 19 and 39 mm respectively, and the curve is divided arbitrarily at 28 mm, giving two groups of scallops, a majority with small, and a minority with large, first growth-bands. Of 4379 scallops examined, 4049, or 92·5% had small, and 330, or 7·5% had large, first growth-bands.

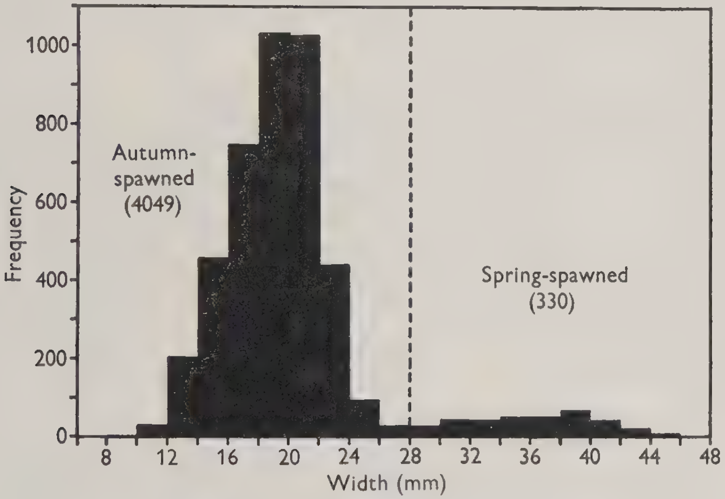

Fig. 3. Width-frequency of the first growth-band.

The two types of first growth-band can be correlated with the breeding cycle of the scallop. *P. maximus* in Manx waters has two main spawnings each year, a spring spawning in April or May and an autumn spawning in late August or September, while there is a small summer spawning in July or early August. Growth of the scallop ceases in December, and the resumption of growth in spring results in the appearance of the growth-ring (see later, p. 485). Spring-spawned scallops would thus grow for a greater length of time before the first cessation of growth than would autumn-spawned individuals. It is suggested that most of the scallops forming the minor group, those with large first growth-bands, arise from the spring spawning, and that most of those forming the major group, those with small first growth-bands, arise from the autumn spawning, while a few of the latter probably arise from the small summer spawning.

Although there are many possible factors which can influence the success of a brood, the difference in the numbers of scallops constituting the two groups can to some extent be accounted for by the amount of spawn released in each spawning. Only those which have just deposited, or are about to deposit, their fourth or any subsequent growth-ring, take part in the spring spawning, and their gonads become only partially spent. On the other hand, mature scallops of all ages (those with two or more growth-rings) take part in the autumn spawning, and the gonads most often become completely spent. Thus more gametes are shed in the autumn than in the spring spawning.

Scallops with large first growth-bands will therefore be called 'spring-spawned' and those with small first growth-bands 'autumn-spawned'.

Tang (1941) did not mention the two types of first growth-band. He measured the distances between the growth-rings of 128 individuals, each of which had ten growth-rings, and found that the width of the first growth-band varied from 10·0 to 29·0 mm, with a mean of 19·0 mm. In the present study, the range obtained was 9 to 45 mm, with a mean of 20·3 mm. Gibson (1956), however, has recently reported the occurrence of two modal groups in the width of the first growth-band of Irish scallops, which he also connects with two peaks of spawning.

THE ANNUAL GROWTH RATE

There is a high degree of correlation between the length, breadth and thickness of the shell of *P. maximus*. The coefficients of correlation were worked out on 614 scallops of all ages from 0+ to 13+. The coefficient of correlation between length and breadth is 0·9937, and that between length and thickness is 0·9597. Furthermore, a high degree of correlation exists between the length of the scallop and the breadth of the flat valve. The coefficient of correlation, worked out on 414 scallops of all ages from 0+ to 13+, is 0·9954. The scallop, in fact, grows proportionately in all dimensions, and retains virtually the same shape throughout its life, with the exception of a concavity on the upper valve during the first year or so. The annual increment of any one of these dimensions will, therefore, give a reliable indication of the rate of growth from one year to another.

Growth curves for *P. maximus* have been drawn, using Bradda and Bay Fine scallops, from the two following measurements: (i) position of successive annual growth-rings on the flat valve, and (ii) annual increase in length, breadth and thickness of the whole shell.

(i) Since each growth-ring represents the position of the edge of the shell at the end of an annual growth period, it is possible to measure directly on the shell of any scallop, the breadth of the flat valve of that scallop at the end of each growth period in its life. By measuring the distances of the various growth-rings from the umbo it is possible to draw up a growth curve. The results are shown in Table 1 and Fig. 4. Only the first eleven rings were measured in autumn-spawned scallops because of the crowding together of the later rings and the small numbers of older scallops obtained. Few spring-spawned scallops were found with more than seven rings.

TABLE 1. MEAN DISTANCES OF THE GROWTH-RINGS FROM THE UMBO (FLAT VALVE)

		Growth-rings										
		1	2	3	4	5	6	7	8	9	10	11
Autumn-spawned scallops	Number measured	4049	3519	2791	1888	1130	526	207	132	67	40	31
	Mean distance (mm)	19·0	48·0	76·4	94·6	104·9	112·3	114·2	119·2	121·6	123·9	126·1
Spring-spawned scallops	Number measured	330	247	141	60	32	25	18	—	—	—	—
	Mean distance (mm)	36·2	65·8	88·1	101·8	109·1	113·4	114·6	—	—	—	—

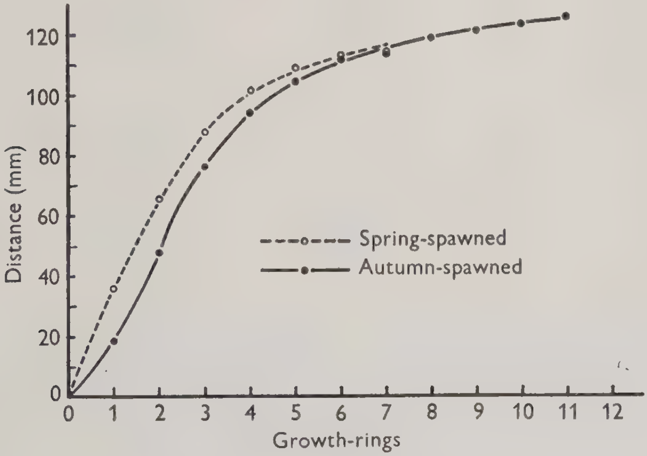

Fig. 4. Mean distances of the growth-rings from the umbo (flat valve).

(ii) Growth curves were drawn by plotting the length, breadth and thickness of scallops measured during the cessations of growth in the winters of

1950–51 and 1951–52; figures for the two winters were combined. No scallop was measured which showed new growth at the edge of the shell. In this method, a scallop which has no growth rings has completed its first growth period, one with one ring has completed two growth periods, and so on. Autumn-spawned scallops with eleven or fewer growth-rings and spring-spawned scallops with seven or fewer rings were used. The results are given in Table 2 and Fig. 5.

TABLE 2. LENGTH, BREADTH AND THICKNESS DURING THE CESSATION OF GROWTH OF AUTUMN- AND SPRING-SPAWNED SCALLOPS

(Data from small-mesh samples, December 1950–March 1951 and December 1951–April 1952, supplemented in the older age-groups by data from commercial samples.)

No. of completed growth-bands	No. measured	Mean length (mm)	Mean breadth (mm)	Mean thickness (mm)
		Autumn-spawned scallops		
1	4	21·2	20·0	5·2
2	173	53·5	50·1	14·7
3	574	87·7	80·8	23·4
4	361	108·1	98·5	28·7
5	330	118·6	107·3	31·2
6	245	128·0	114·9	34·0
7	103	131·8	117·7	35·2
8	40	136·8	122·8	36·0
9	40	137·9	124·0	36·9
10	16	142·8	127·2	38·9
11	14	142·1	126·8	38·6
12	8	148·1	134·4	41·8
		Spring-spawned scallops		
1	28	37·5	35·7	10·4
2	49	73·3	67·7	19·1
3	64	98·0	90·0	26·0
4	48	114·7	104·8	30·8
5	11	118·9	107·0	30·7
6	8	134·4	120·6	35·8
7	3	134·2	115·8	36·3
8	6	140·8	124·2	36·3

The second method has several disadvantages. The dimensions at the end of a particular growth period can be obtained only by measuring scallops of that particular age; it is dependent on small-mesh dredge samples for younger scallops, and even then few are caught (see later, p. 490); and it must use scallops caught during the cessation of growth. In the first method, on the other hand, all the growth-rings can be measured on every shell; scallops from the commercial dredge can be used, since information about the early years of the scallop's life can be obtained from older shells; and scallops caught at any time of the year can be used.

In the first method, data from scallops of different year-classes are grouped together, thereby masking any variation in growth rate there may be from year to year. Such variation could be shown by measuring one particular growth-band, say the third, in scallops of all ages in one particular season.

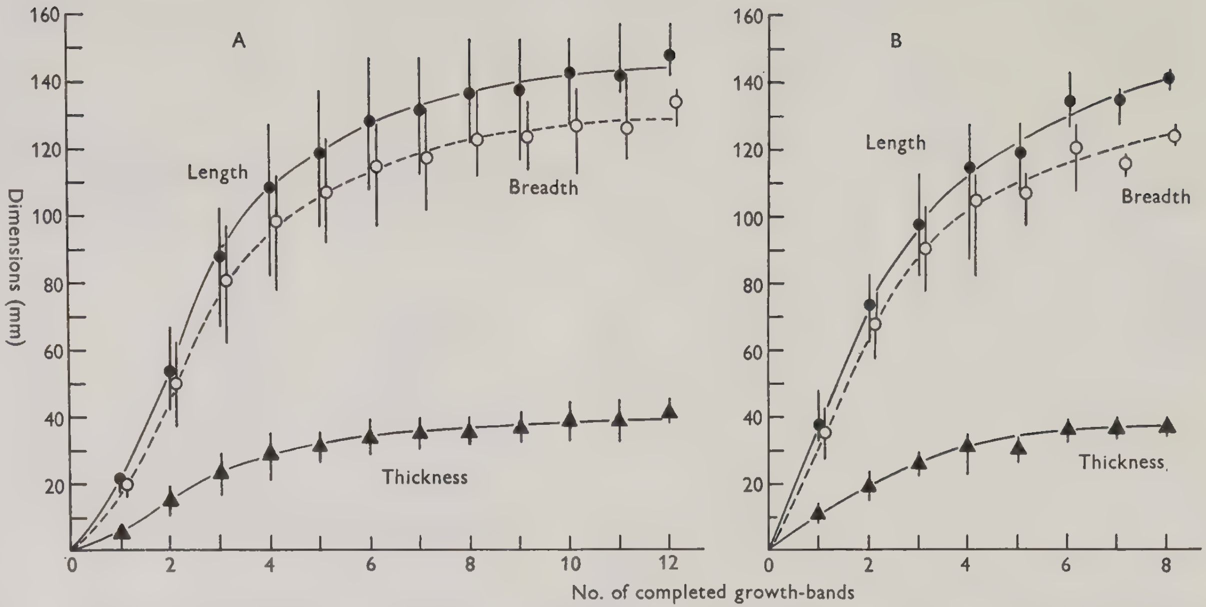

Fig. 5. Length, breadth and thickness of scallops of various ages measured during the cessation of growth. Vertical lines represent the range of sizes in each age-group. Data for breadth are displaced slightly to avoid confusion with length data. (A) Autumn-spawned scallops. (B) Spring-spawned scallops.

The second method would also afford a means of comparing the rates of growth in different years if sufficient scallops were measured to allow of keeping the figures for various winters separate.

The growth curves show that, in autumn-spawned scallops, growth is greatest in the second and third annual growth periods, and is approximately equal in extent in these periods. Thereafter the annual growth decreases progressively. Spring-spawned scallops, which are larger in all dimensions at the end of the first growth period than autumn-spawned scallops, grow most in the first two periods, in each of which growth is comparable with that of autumn-spawned scallops in the second or third period. The annual increment decreases steadily in each growth period after the second. The mean dimensions of spring- and autumn-spawned scallops approach each other more closely with increasing age.

The growth curves obtained by the two methods are in good agreement, and are of a type characteristic of lamellibranch shells (Fairbridge, 1953). Similar curves have been obtained for the American Giant Scallop, *Placopecten* (*Pecten*) *grandis*, by Stevenson (1934) and Posgay (1953); for the Tasmanian commercial scallop, *Notovola meridionalis*, by Fairbridge (1953); and for *Pecten maximus* in Ireland by Gibson (1956).

A large range of sizes is covered by each age-group (see Fig. 5); it is possible, for instance, to find a 4+ scallop larger than an 8+ one in the same sample. This also is a characteristic of the growth of lamellibranchs (Fairbridge, 1953).

COMPARISON OF THE RATES OF GROWTH OF SCALLOPS FROM DIFFERENT DEPTHS

Comparisons were made of the rates of growth of scallops from the five areas (p. 473) from which either regular or occasional samples were taken. This was done by measuring the mean distances from the umbo of the growth-rings on the flat valve of the shell. Spring- and autumn-spawned scallops were considered together, since the former are so few in number as to make little difference to the result.

The results are shown in Table 3 and Fig. 6.

The growth rates can be placed in order, from greatest to least, to correspond with the order of increasing depth of water:

Station (No. and name)		Depth of water (fm. and m)
3	Gob-yn-Ushtey	9–11 (16·5–20·1)
1	Bay Fine	13–16 (23·8–29·3)
2	Bradda	
4	The Breast	19–20 (34·7–36·6)
5	W.S.W. of Chicken Rock	28–29 (51·2–53·0)

Differences in rate of growth between localities are characteristic of lamellibranchs (Fairbridge, 1953). Several factors have been suggested to account for local differences of growth rate in pectinid and other molluscan species,

TABLE 3. MEAN DISTANCES FROM THE UMBO OF GROWTH-RINGS ON THE FLAT VALVE OF THE SHELL

(Spring- and autumn-spawned scallops combined.)

		Growth-rings									
		1	2	3	4	5	6	7	8	9	10
Bay Fine (Stn. 1)	Number measured	1977	1732	1385	1013	633	277	108	51	22	12
	Mean distance (mm)	19·6	48·4	76·6	94·8	105·2	112·7	118·3	123·1	125·4	128·4
Bradda (Stn. 2)	Number measured	2402	2034	1547	935	529	274	117	90	51	31
	Mean distance (mm)	20·8	49·9	77·2	94·7	104·7	112·0	110·4	116·9	119·5	121·9
Gob-yn-Ushtey (Stn. 3)	Number measured	117	117	100	96	91	75	62	40	11	10
	Mean distance (mm)	22·3	54·8	83·9	102·3	112·6	118·4	122·0	124·6	129·9	132·0
Breast (Stn. 4)	Number measured	116	116	114	100	88	48	28	22	11	5
	Mean distance (mm)	19·4	46·0	70·4	87·5	96·7	103·2	109·3	112·3	114·7	117·5
Chicken (Stn. 5)	Number measured	100	100	100	100	100	93	85	58	28	27
	Mean distance (mm)	18·7	39·8	62·4	82·0	95·1	101·8	105·5	108·9	110·7	111·5

including currents (Gutsell, 1930; Fairbridge, 1953), temperature (Coe & Fox, 1944) and the nature of the sea bed (Fairbridge, 1953).

Gibson (1956) found that scallops living on sheltered beds grew more quickly than those on exposed beds, and suggested that this is due to excessive particle bombardment interfering with feeding on the latter beds. This could not be the cause of the differences in growth rate between different areas in the present study, since all the areas were exposed.

In the present study, scallops living in the shallowest water were found to grow more quickly than those in deeper water. It is suggested below (p. 489) that the growth of *P. maximus* is influenced by temperature. It is of interest

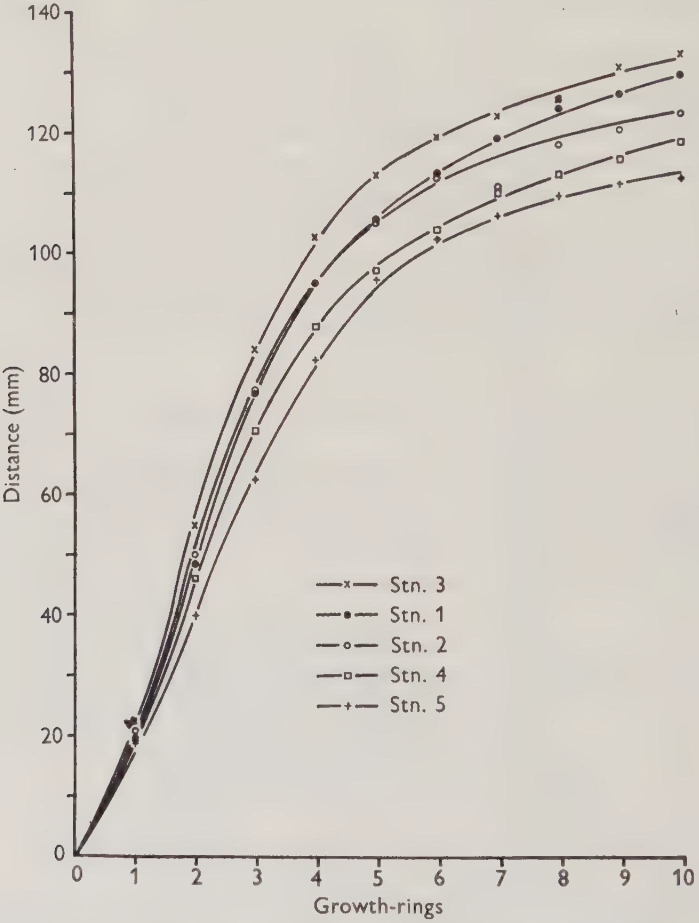

Fig. 6. A comparison of the rates of growth of scallops from five areas (see chart, Fig. 1). The graph shows the mean distances from the umbo of the growth-rings on the flat valve of the shell. Data for spring- and autumn-spawned scallops are combined.

in this connexion that from May to August, when the scallop is growing most rapidly, bottom temperatures inshore are higher than those offshore,[1] probably resulting in a higher rate of growth on the shallower, inshore beds.

THE ANNUAL PERIOD OF GROWTH

The period of growth was determined by measuring throughout the year the width of shell outside the outermost growth-ring on the flat valve of the shell of Bradda scallops, spring- and autumn-spawned scallops being treated separately. Autumn-spawned scallops with 0–5 growth-rings were used, but insufficient spring-spawned scallops were obtained with more than three rings. Since the annual growth-ring is laid down some time in March, April or May, it is necessary to consider the period March 1951–May 1952 in order to cover a complete growth period. The monthly measurements are, where necessary, reinforced by data from the corresponding month of the other year. Samples taken in the small-mesh dredges were used, supplemented in the larger sizes by scallops from commercial samples.

The results (Table 4 and Fig. 7) show that growth commences in March, April or May, and is most rapid from June to September or October, when it begins to slow down, stopping altogether from December until the following March, April or May. These results agree well with those of Gibson (1956), who found that growth of *P. maximus* in Irish waters ceases from November to February.

With two exceptions, all the scallops which had not yet deposited their first growth-ring were obtained between December and May, after the completion of their early growth. The exceptions were two scallops, 3·5 and 3·0 mm long, which were found on the Bradda bed in August 1952. These probably arose from the small spawning of July 1952, but since this is not certain they were not included in Table 4 and Fig. 7. No suggestion can be advanced at present to explain my inability to find more young specimens; Elmhirst (1945) found many between 0·7 and 4·0 mm attached to *Laminaria saccharina* in the Firth of Clyde in July and August.

POSSIBLE FACTORS CAUSING CESSATION OF GROWTH AND THE DEPOSITION OF THE ANNUAL RING

The growth of an animal is the total result of many interacting factors, of which temperature is well known to be important. With scallops, Belding (1931) found that growth of *Pecten irradians* ceased during the cold winter months, and was resumed in May when the temperature of the water had reached 45–50° F (7–10° C). But other factors may have to be taken into account—for instance alternate scarcity and abundance of food (see Thompson, 1942). It has been suggested that in *P. irradians*, cessatian of growth is caused in

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[1] Data kindly provided by Mr D. J. Slinn. The temperatures were taken on the Bay Fine bed and at a position near the Chicken Rock bed (Station 5).

TABLE 4. GROWTH OF SHELL (IN MM) BEYOND THE LAST-FORMED ANNUAL RING, THROUGHOUT THE YEAR

Number of growth-rings		M.	A.	M.	J.	J.	A.	S.	O.	N.	D.	J.	F.	M.	A.	M.
	Autumn-spawned scallops, age-groups 0 to 5															
0	Number measured	—	—	—	—	—	—	—	—	—	1	1	2	—	—	4
	Mean growth	—	—	—	—	—	—	—	—	—	19·5	15·5	20·5	—	—	22·3
1	Number measured	—	—	—	4	—	2	3	3	9	9	6	12	31	7	—
	Mean growth	—	—	—	9·5	—	22·5	24·0	26·0	30·4	31·9	30·7	31·2	30·1	31·3	—
2	Number measured	1	27	38	20	15	8	9	7	35	5	9	50	5	37	12
	Mean growth	1·0	2·2	4·9	7·9	12·7	18·8	21·3	25·0	27·5	28·4	30·1	30·4	30·4	30·9	30·0
3	Number measured	3	24	74	127	36	17	80	66	52	19	45	90	49	45	9
	Mean growth	2·0	1·9	2·9	4·6	7·6	11·5	14·1	15·9	17·8	18·8	17·8	18·0	18·6	18·9	18·5
4	Number measured	—	29	49	52	16	8	13	24	9	8	16	12	13	5	22
	Mean growth	—	2·0	1·7	2·3	5·2	6·3	9·4	10·4	12·2	11·4	11·6	12·2	12·0	13·6	10·7
5	Number measured	—	3	12	58	43	27	22	20	32	9	35	22	21	33	16
	Mean growth	—	0·3	1·5	1·6	3·3	4·4	5·0	6·2	7·5	7·1	7·3	6·8	7·8	7·8	7·3
	Spring-spawned scallops, age-groups 0 to 3															
0	Number measured	—	—	—	—	—	—	—	—	—	—	1	2	13	—	2
	Mean growth	—	—	—	—	—	—	—	—	—	—	30·0	33·5	34·2	—	36·5
1	Number measured	—	1	1	1	—	—	—	5	7	8	2	13	8	5	—
	Mean growth	—	4·0	3·0	10·0	—	—	—	25·2	26·6	30·4	29·5	31·2	28·1	33·0	—
2	Number measured	1	19	10	6	5	12	7	5	2	—	11	6	32	13	—
	Mean growth	1·0	2·5	4·7	7·3	9·4	15·8	17·6	19·8	21·5	—	22·5	20·8	21·7	23·7	—
3	Number measured	—	1	13	10	6	1	12	17	15	6	8	9	7	8	2
	Mean growth	—	1·0	1·8	3·0	6·8	8·0	10·7	10·3	12·1	14·0	13·1	12·1	12·9	14·5	15·0

Rhode Island waters by spawning (Risser, 1901), and in North Carolina waters by gonad development (Gutsell, 1930). Gonad development is also suggested in *Chlamys varia* (Dalmon, 1935) and in *Notovola meridionalis* (Fairbridge, 1953). Tang (1941), working on *Pecten maximus* at Port Erin, assumed that the growth-ring which was laid down in April, May or June represented not the resumption but the cessation of growth. Finding more than 50% of scallops with running gonads in these months, he stated, erroneously, that the cause

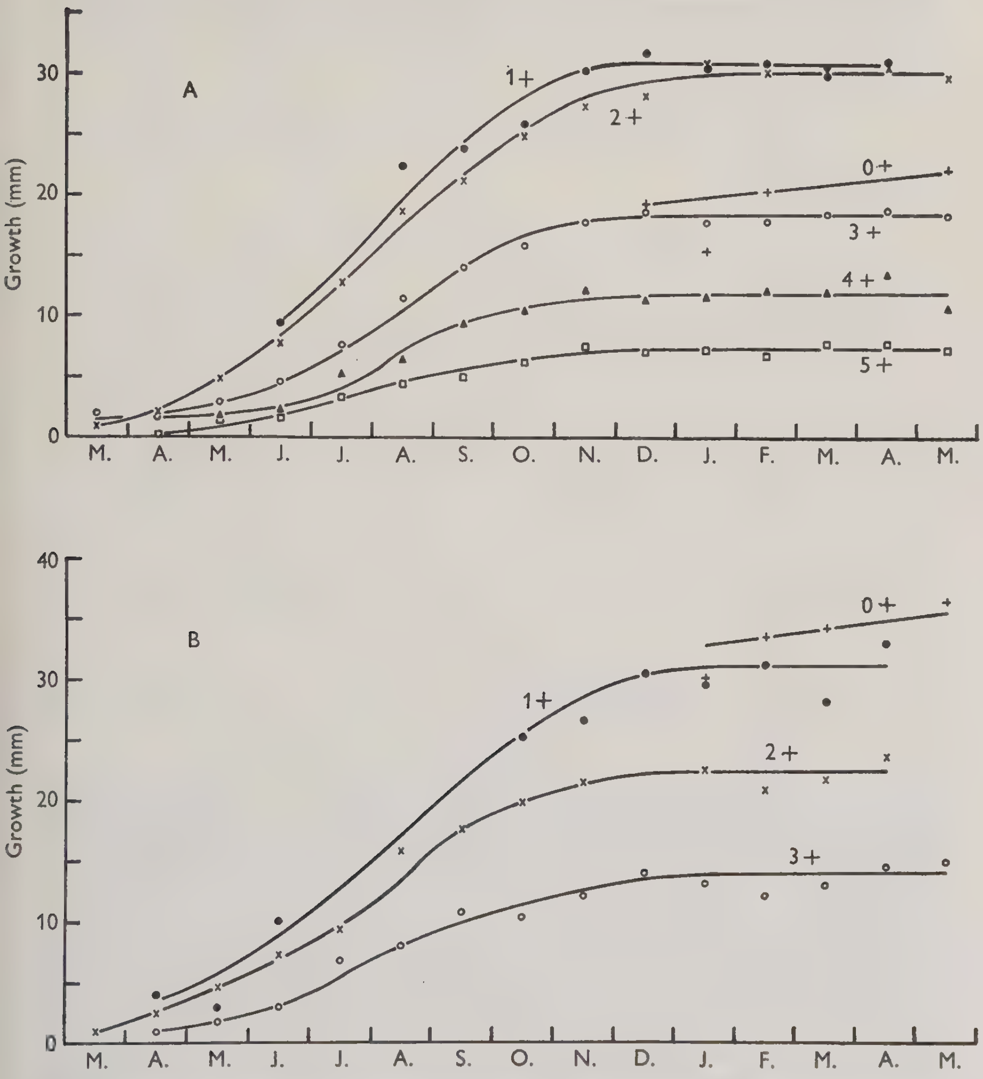

Fig. 7. Growth of shell (in mm) beyond the last-formed annual ring, throughout the year. (A) Autumn-spawned scallops, age-groups 0–5. (B) Spring-spawned scallops, age-groups 0–3.

of cessation of growth may be correlated with the poor condition after the formation of gametes and spawning.

Spawning cannot be an effective factor in causing the cessation of growth of *P. maximus*, since spawning occurs in April or May and in July, August and September, all during the period of growth, while growth ceases from December to March, during which period there is no spawning. Spawning cannot possibly be responsible for the cessations of growth in the animal's first two winters, since scallops do not spawn for the first time until the summer after the deposition of the second growth-ring (Mason, 1953).

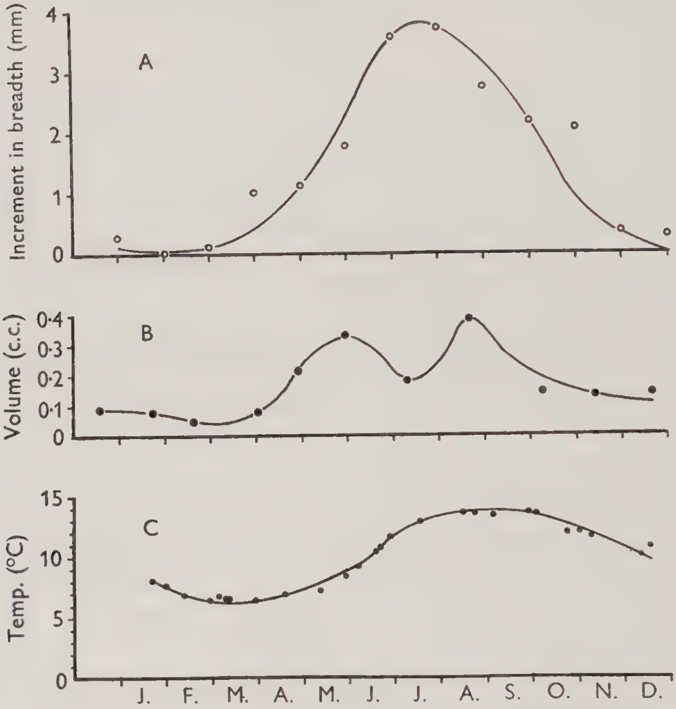

Fig. 8. (A) Mean monthly increment in width of the flat valve in 2-, 3- and 4-group scallops combined. (B) Volume of solid matter in stomachs of scallops, 1952. All scallops were of the 1948 brood. (C) Bottom temperatures on the scallop beds (Stations 1 and 2), 1951.

The relation between the seasonal growth of *P. maximus*, the rate of feeding, and the bottom sea temperature over the scallop beds, is shown in Fig. 8. The seasonal growth is shown by the monthly mean increment in width of the flat valve, and the rate of feeding by the volume of solid matter (largely diatoms, dinoflagellates and detritus) in the stomach. During the cessation of growth, the sea temperature is at its annual minimum, and the rate of feeding is at its lowest. The latter may be due to cold, to lack of available food, or to both. Gonad development occurs throughout the year, but most slowly in winter, possibly owing to low temperature, low rate of feeding, or both.

It is likely that several factors act together to cause the annual cessation of growth of *Pecten maximus*. The following hypothesis is tentatively suggested. During the cold of winter, body processes occur slowly, and feeding is sufficient for slow gonad development, but not growth, to occur. With the higher temperature of summer the body processes are speeded up, and feeding is sufficiently high for more rapid gonad development and also growth to occur, even during the slight drop in feeding in July. (Too much significance should probably not be attached to the July minimum, as only one sample of stomach contents was obtained between May and August.)

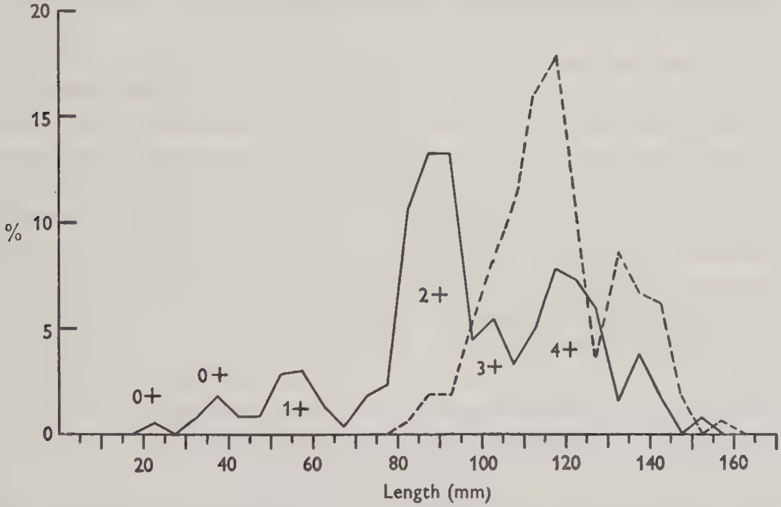

Fig. 9. Length-frequency of Bradda scallops in samples; March 1951. R.V. *William Herdman*. Length in 5 mm groups. ——, 4 ft. small-mesh dredge (398 scallops measured); – – –, 6 ft. commercial dredge (161 scallops measured).

LENGTH-FREQUENCY AND THE SCARCITY OF YOUNG SCALLOPS

Comparisons were made of the length-frequencies of scallops taken in the 6 ft. commercial and 4 ft. small-mesh dredges (see above, p. 473) on the Bradda bed by R.V. *William Herdman* in March 1951 (Fig. 9). The samples were made up of the contents of several dredge hauls, each of 15 min duration in the case of the commercial dredge, and of 7 min duration in the case of the small-mesh dredge, which quickly filled with gravel and shells.

The commercial dredge, which is typical of Manx commercial scallop dredges, exercises a selective action, which begins to act at 95–100 mm (Fig. 9). There is no selection of scallops more than 100 mm long, but few less than 95 mm long are taken. None less than 80 mm long was caught in

March 1951, but the commercial dredge occasionally retained a few smaller scallops among the stones and shells in the bag. On the average the scallop attains a size of 100 mm late in its fourth growth period, so that most scallops spawn in at least two years before they can be caught.

The length-frequency curve for the small-mesh dredge shows a number of peaks, of which the first few each consist entirely or almost entirely of scallops of one age-group (Fig. 9). The most abundant age-group in the sample was that composed of scallops with two growth-rings. It would be expected that the youngest scallops would be most abundant, decreasing in abundance with increasing age. The contrary results appeared to imply that a proportion of the younger, and therefore smaller, scallops, was being missed.

A scarcity of young scallops in dredge samples was noted also by Priol (1930) and Baird (1952), and Baird & Gibson (1956) suggested two possible causes. (i) Settlement may occur on feeder beds, probably inshore, from which young scallops migrate back to the parent beds on attaining a certain size or age; and (ii) young scallops may be present on the beds, but for some reason are not caught. Baird & Gibson (1956), by direct observation on the scallop beds by diving, found that the scallops showed little escape reaction. They compared collections of scallops obtained (*a*) by means of a small-mesh dredge, and (*b*) directly by diving, on the beds off Port Erin, and found that diving yielded much smaller sizes and lower age-groups than dredging. This was not due to mesh-selection of the dredge-bag, since it was lined with shrimp netting which would retain scallops of all sizes. Baird & Gibson concluded, therefore, that the teeth, which are the only other selective feature of the dredge, must be the effective selection agent, but that selectivity is continued beyond the point which might reasonably be expected from mesh-size and tooth-spacing. (My own commercial dredge sample throws no light on this discovery owing to the small difference between tooth-spacing, 89 mm, and the diameter of the rings in the belly of the bag, 82 mm.) In the present study the teeth of the small-mesh dredge must have dug deep into the sea bottom, since the bag rapidly became full of fine gravel, shells, stones and other objects. This means that the teeth were ineffective as a selective agent, and the dredge acted as a complete bottom-sampler. Even so it collected less than the expected proportion of young (0+ and 1+) scallops. In fact my small-mesh dredge sample compared closely with the sample collected by hand, from visual observation, by Baird & Gibson. It would appear, therefore, that fewer 0+ and 1+ scallops than expected are actually on the beds.

As Baird & Gibson pointed out, few 0+ scallops would be expected in their collections, which were taken in July 1953, since 90–95 % of scallops at Port Erin are autumn-spawned. It is still difficult, however, to account for the relative shortage of 1+ scallops in Baird's & Gibson's collections and of 0+ and 1+ scallops in my small-mesh dredge samples taken in March and at all other times of the year.

This paper is based on parts of a thesis[1] presented to the University of Liverpool in 1953 for the degree of Ph.D. The work was carried out at the Marine Biological Station, Port Erin, while I held a Herdman Studentship and Liverpool University Research Fellowship. I wish to thank the Director, Mr J. S. Colman, for suggesting the problem to me, for his constant guidance and valuable criticisms, and for reading the manuscript. Mr A. B. Bowers and Mr A. D. McIntyre also read the manuscript and made helpful suggestions. I am grateful for the help given by the scientific staff, students and technical staff of the Marine Biological Station, particularly by Mrs D. I. Williamson and Miss M. MacLeod. Lastly, I wish to thank the Masters and crews of the R.V. *William Herdman* and the M.B.'s *Cypris* and *Runa*.

SUMMARY

Growth-rings on the shell of the scallop (*Pecten maximus*) are laid down annually, in spring, and so can be used to determine the age.

Scallops grow from spring to December, and cease growing in winter. The resumption of growth in the spring is marked by the appearance of the annual growth-ring at the edge of the shell.

The first year's growth is of one of two types. A few scallops have shells which show a large first year's growth, 28 mm or more wide, while the great majority have shells which show a small first year's growth, less than 28 mm wide. These two types probably depend on the two principal spawning periods of the scallop, most of the former arising from the spring spawning and most of the latter from the autumn one.

The growth curve of *P. maximus* is of a type characteristic of lamellibranch shells. The annual growth is greatest in the first two or three years of life, after which it decreases steadily.

Scallops grow more quickly in shallow water than in deeper water.

Possible causes of the annual cessation of growth are discussed.

A paucity of young scallops was noted in the dredge samples, as previous workers have reported. No reason can be given.

REFERENCES

Baird, R. H., 1952. The English Channel escallop beds. *Fishing News*, No. 2064, p. 9.

Baird, R. H. & Gibson, F. A., 1956. Underwater observations on escallop (*Pecten maximus* L.) beds. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 555–62.

Belding, D. L., 1910. *A Report upon the Scallop Fishery of Massachusetts*. Boston: Commonwealth of Massachusetts.

—— 1931. *The Scallop Fishery of Massachusetts*. Boston: Comm. Mass., Dept. Cons. [An abbreviated reprint of the 1910 paper.]

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[1] 'Investigations on the scallop [*P. maximus* (L.)] in Manx waters', 1953.

Coe, W. R. & Fox, D. L., 1944. Biology of the California sea-mussel (*Mytilus californianus*). III. Environmental conditions and rate of growth. *Biol. Bull., Woods Hole*, Vol. 87, No. 1, pp. 59–72.

Coker, R. E., Shira, A. F., Clark, H. W. & Howard, A. D., 1921. Natural history and propagation of freshwater mussels. *Bull. U.S. Bur. Fish.*, Vol. 37, pp. 75–181.

Dakin, W. J., 1909. *Pecten. L.M.B.C. Mem.*, No. 17.

Dalmon, J., 1935. Note sur la biologie du pétoncle (*Chlamys varia* L.). *Rev. Trav. Off. Pêches marit.*, T. 8, pp. 268–81.

Elmhirst, R., 1945. Clam fishing in the Firth of Clyde. *Trans. Butesh. nat. Hist. Soc.*, Vol. 13, pp. 113–16.

Fairbridge, W. S., 1953. A population study of the Tasmanian 'commercial' scallop, *Notovola meridionalis* (Tate) (Lammellibranchiata, Pectinidae). *Aust. J. mar. freshw. Res.*, Vol. 4, No. 1, pp. 1–40.

Gibson, F. A., 1956. Escallops (*Pecten maximus* L.) in Irish waters. *Sci. Proc. R. Dublin Soc.*, Vol. 27, pp. 253–70.

Gutsell, J. S., 1930. Natural history of the bay scallop (*Pecten irradians*). *Bull. U.S. Bur. Fish.*, Vol. 46, pp. 569–632.

Posgay, J. A., 1953. The sea scallop fishery. *6th Report on Investigations of the Shellfisheries of Massachusetts.* Boston: Comm. Mass., Dept. nat. Resour. Div. mar. Fish.

Priol, E., 1930. La coquille Saint-Jacques (*Pecten maximus*). *Rev. Trav. Off. Pêches marit.*, T. 3, pp. 143–73.

Risser, J., 1901. Habits and life-history of the scallop (*Pecten irradians*). *Rep. R. I. Comm. inl. Fish.*, Year 31, pp. 47–55.

Stevenson, J. A., 1934. The growth-rate of Canadian scallops. *Progr. Rep. Atl. biol. Sta.*, No. 11, pp. 10–11.

Tang, S.-F., 1941. The breeding of the escallop [*Pecten maximus* (L.)] with a note on the growth rate. *Proc. Lpool biol. Soc.*, Vol. 54, pp. 9–28.

Thompson, D'A. W., 1942. *On Growth and Form.* 1116 pp. Cambridge University Press.

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SEA CURRENTS OFF THE NORTHUMBERLAND COAST

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(Text-fig. 1)

The non-periodical currents off the southern Northumberland coast are in general weak and variable and can with safety be ignored by the prudent navigator. For the student of the region's ecology, however, they are of greater significance, and it is of some importance that as exact a knowledge as possible of their speed and direction should be available. The present account is concerned with water movements close to the surface a mile or so offshore; deep currents, it is hoped, will form part of a separate study.

Earlier investigations have usually formed part of much wider surveys in the North Sea and the results have tended to be rather generalized for the small region between the River Tyne entrance (55° 01′ N., 1° 24′ W.) and Newbiggin Point (55° 11′ N., 1° 30′ W.) at present under consideration. Perhaps in consequence of this, different workers have produced conflicting results, although the work of Tait in particular has shown (Tait, 1937) that there may be considerable seasonal and even yearly fluctuations in the currents of the northern North Sea.

The main question at issue is whether the residual current flows along the Northumberland coast in a southerly or a northerly direction. A few instances of the differing conclusions reached will illustrate this. Fulton (1897), deriving his conclusions from drift bottle recoveries, held that it ran to the southward. Böhnecke, quoted by Meek (1926), and basing his conclusions on salinity curves, believed that it ran to the northward. Meek himself, in a paper in which he described the charting of the North Sea currents as 'the most fundamental problem of all' (in relation to fisheries biology), strongly refuted Böhnecke's conclusions. Like Fulton he believed in a southerly current and offered in evidence replies to a letter he sent to fishery officers, etc., up and down the coast inquiring whether, in their region, the ebb or flood ran the stronger. This appeal to common observation brought him a response from Northumberland stating that 'the flood tide is the stronger, the current going in a southerly direction'. But he failed to ask an equally important question, whether the ebb or flood ran the longer, and his argument is the weaker for this omission.

Tait's results, based on numerous drift bottle returns, were more complex.

His final chart (1937) indicated a southerly drift about 15 miles offshore and a northerly counter-current running closer inshore. However, he remarked earlier (1930) that the correspondence between superficial water displacement and wind direction and velocity, particularly in shallow water areas adjacent to land may at times be very close. Since the prevailing winds on the Northumberland coast are westerlies this suggests, allowing for Coriolis force, a mean vector current in the shallow, coastal waters running approximately south-east.

In view of these differing accounts it is difficult to decide from the literature alone what is the true nature of the residual currents off Northumberland. In April 1956 I began a survey in the hope of providing sufficient information from purely local waters to solve this problem. The method I employed was the old and well tried one of following the movements of a free-floating buoy having little windage, and plotting its track. As in all direct methods of measuring currents in the North Sea it was necessary for each observation to extend over a period of at least half a lunar day so that tidal effects could be eliminated. Observations were begun at various states of tide and continued for between 12 and 13 h, the exact period to be spent tracking the buoy being determined from an inspection of the tide tables.

The buoy used was a fisherman's dan buoy to which was attached a drogue to reduce leeway. During the work a number of drogues were lost and because of this they were progressively strengthened in construction. In particular it was found that the connexion between buoy and drogue was subjected to considerable stress in heavy weather, which broke wires and opened shackles. In its most recent form (Fig. 1) the buoy had spliced round it a short sling of 2 in. rope and to this sling was shackled 14 ft. of 1 in. circumference wire. The wire was in turn shackled to an eyebolt in the bottom of the drogue. The drogue itself was in the form of an open ended box whose framework consisted of four wooden uprights each 4 ft. 6 in. by 2 in. by 2 in., and diagonal bars of steel 5 ft. long and $\frac{1}{2}$ in. in diameter at each end. Details of these diagonals are shown in Fig. 1. The drogue was given a covering of no. 4 canvas.

The effective depth at which the current was measured by this instrument was assumed to be about 12 ft., i.e. the depth of the mid-point of the drogue. The effects of water movement on the buoy and wire were disregarded since it was felt that they would not be significantly different from those acting on the drogue. Consideration was, however, given to the effect of wind action on the buoy and this will be referred to later. In fact, the effect was negligible.

At sea the buoy's position was fixed every 45 min. by horizontal and vertical sextant angles of conspicuous marks ashore. At the time of each fix the force and direction of the wind were estimated, short notes were made on the weather and state of the sea, and a sounding was taken. Owing to the proximity of the coast positions were accurate to within at least 0·1 mile and usually much better.

From April 1956 nineteen complete runs were made with the free-floating buoy over a period of 12 months. The incomplete run of 4 December 1956 has also been included, an estimate being made of the last 2 h of the run. The observations were spread as evenly as possible over the year and at least one run was made in each month except February 1957. As far as practicable the work was conducted in all states of weather; it should be noted, however, that projected runs were cancelled on days with strong north-easterly winds when the Northumberland coast becomes a lee shore.

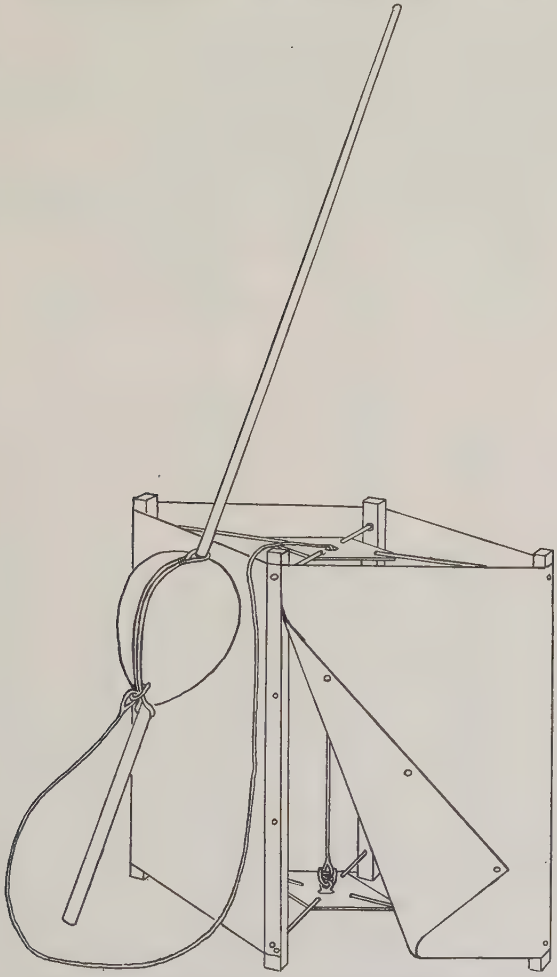

Fig. 1. Diagram of the buoy and drogue used in the investigations. A flap of canvas is raised to show the attachment of the wire to the drogue.

From the results obtained (Table 1) certain values may immediately be calculated. First, the mean vector current or resultant direction and speed works out at 114° and 1·30 miles per half lunar day or about 0·1 knot. Secondly, the mean vector wind during the observations is 273°, 5·4 knots. Thirdly, the predominant direction and speed of current is 120° and 2·5 miles per half lunar day, or about 0·2 knot. The predominant current has been calculated, following Meteorological Office practice, as follows: observations are totalled in successive 90° sectors around the whole compass, each sector being displaced 15° from its predecessor. The mid-direction of the sector containing the largest number of observations is the predominant direction. The speed is the average speed of all currents observed flowing within 45° on either side of the predominant direction. The predominant current refers to the current most likely to be encountered at any one time, while the mean vector current refers to the overall movement of water over a period.

TABLE 1. WIND AND CURRENT OBSERVATIONS DURING THE YEAR

Directions are true and measured from north throughout this account

						Winds in preceding 24 h	
Date 1956–7	Set (°)	Drift (miles)	Downwind direction (°)	Downwind speed (knots)	Wind-current angle (°)	Downwind (°)	Speed (knots)
3 Apr.	159	3·7	135	19·0	+ 24	090	4·1
16 Apr.	102	1·5	035	2·5	+ 67	076	2·6
20 Apr.	359	1·9	313	8·0	+ 46	339	3·3
25 Apr.	189	1·3	189	5·4	0	221	3·8
2 May	335	0·5	326	5·8	+ 9	011	4·0
3 May	075	1·0	018	7·4	+ 57	027	2·8
14 June	179	0·5	190	3·1	− 11	124	6·7
19 June	137	5·6	086	15·8	+ 51	054	6·9
24 July	003	0·8	218	1·0	+145	089	5·1
27 July	351	1·8	274	1·8	+ 77	097	11·9
7 Aug.	141	3·1	124	1·4	+ 17	263	1·3
9 Aug.	061	1·0	214	4·6	−153	088	6·5
13 Sept.	138	3·0	106	13·7	+ 32	090	6·5
23 Oct.	082	2·1	069	10·7	+ 13	050	11·4
26 Oct.	156	1·7	130	14·0	+ 26	136	17·3
13 Nov.	071	1·1	045	7·1	+ 26	115	8·0
4 Dec.	099	3·4	090	27·7	+ 9	090	20·0
10 Jan.	127	1·2	137	11·2	− 10	090	14·7
22 Jan.	087	0·7	003	5·6	+ 84	044	9·7
27 Mar.	118	3·1	061	16·2	+ 57	055	7·6

Interpretation of the results was attempted in various ways. No obvious relationship appears to exist between a current's set and drift and the moon's phase or the tidal range at the time of observation. Similarly, there is no clear relationship between a current and the departure of a tide from its predicted level as measured on a tide gauge at the River Tyne entrance. It remains to be seen whether two tide gauges widely separated would reveal a slope of the sea and hence a current-generating force. Density differences in the region are in general too small to cause horizontal currents of any importance.

The only valid relationship revealed by the results appears to be that between current and wind. Currents are listed in Table 1 against the directions towards which the winds blew (henceforward called downwinds), obtained from the trigonometric summation of the $\frac{3}{4}$-hourly wind observations. Column 6 of this table shows the difference in degrees between current set and downwind, marked positive if the current ran to the right of downwind and negative if it ran to the left. Columns 4 and 6 are rearranged in Table 2 with downwinds increasing clockwise from north. It will be seen that the current ran consistently into the quadrant to the right of downwind except in the five downwind records 137° to 218°; these records are anomalous and will be dealt with later. The remaining currents show a mean deflexion of 40° with standard deviation 26°. The figures are in close agreement with many open ocean records, e.g. at the ocean weather ship station 'I' (Hay, 1954), where eighteen observations, each of 5 days' duration, showed a deflexion of 42° with standard deviation 42°. For comparison, current sets were related to winds recorded during the 24 h preceding each observation, wind data being obtained from 3-hourly readings of an anemometer at Tynemouth. The relationship here was less good, with deflexion 20° and standard deviation 59°. From this it seems likely that the onset of a given wind and the appearance of the induced current are separated in time by only a very few hours.

TABLE 2. DOWNWIND DIRECTIONS AND WIND-CURRENT ANGLE

Downwind (°)	Wind-current angle (°)	Downwind (°)	Wind-current angle (°)
003	+84	130	+ 26
018	+57	135	+ 24
035	+67	137	− 10
045	+26	189	0
061	+57	190	− 11
069	+13	214	−153
086	+51	218	+145
090	+ 9	274	+ 77
106	+32	313	+ 46
124	+17	326	+ 9

A relationship can also be established between wind and current speeds using the data in columns 3 and 5 of Table 1. For all observations $U_s = 1{\cdot}30$ miles per half lunar day, $U_a = 67$ miles per half lunar day, where U_s represents residual current velocity and U_a residual wind velocity.

Following Thorade (Proudman, 1953, p. 176) and employing a mean latitude (ϕ) of 55° 06′ N. we obtain the equation

$$\frac{U_s\,(\sin\phi)^{\frac{1}{2}}}{U_a} = 0{\cdot}018.$$

For this expression Thorade found the value 0·0126 and Ekman 0·019.

There appears to be no basic current independent of wind such as has been found in the southern North Sea from an analysis of lightship current records (Lawford & Veley, 1955).

With regard to the five observations which were excluded from the deflexion calculations, if the mean deflexion is applied to each wind all the sets become onshore, for between the Tyne and Newbiggin the trend of the coast is about north-west to south-east. But only very small onshore sets were recorded; potential onshore sets, then, are deflected by the coastline, those to the south of south-west being deflected anticlockwise and those to the north of south-west clockwise.

Throughout this account it has been assumed that the current observations were made during a fair sample of the wind speeds experienced during the year. Table 3 shows that this is approximately true. Wind-speed estimations made on the 20 sea-going days at 9 a.m. are compared with estimations made at corresponding times at Cullercoats on 237 occasions. Wind directions (Table 4) do not correspond so closely, and it is clear that too many north-west winds and too few south-east winds are represented in the sea observations. This has had a small effect on the figures given for residual and predominant currents; if a residual current is worked for the 237 shore observations and a deflexion of +21° is allowed, as was found for residual winds and currents at sea the result, using Thorade's formula, is 110°, 0·06 knot, which is not very different from the 114°, 0·1 knot already found.

TABLE 3. 9 A.M. WIND FORCES AT CULLERCOATS AND AT SEA, EXPRESSED AS PERCENTAGE OF TOTAL OBSERVATIONS

	At Cullercoats	At sea
Observations ...	237	20
Calm	1·7	0
Force 1	12·2	15
Force 2	21·7	25
Force 3	26·2	20
Force 4	19·4	25
Force 5	10·1	10
Force 6	5·9	0
Force 7	2·1	5

TABLE 4. 9 A.M. WIND DIRECTIONS AT CULLERCOATS AND AT SEA, EXPRESSED AS PERCENTAGE OF TOTAL OBSERVATIONS

	At Cullercoats	At sea
Observations ...	237	20
Calm	1·7	0
000–089°	14·3	10
090–179°	20·2	5
180–269°	28·2	25
270–359°	35·5	60

Finally, an answer must be given to the question of whether the buoy used in the investigations was seriously affected by the wind. It would be simple to discover this by attaching a current meter to the drogue, when the total reading would be due to wind drift, but for the very low speeds (certainly less than 10 ft a minute) which would have to be measured in a horizontal direction alone; so this solution is impracticable at sea. It might have been possible to test the buoy's downwind movement in one of the Tyne docks were it not suspected that the dock water itself is always in slow motion. Short of wind tunnel experiments I was forced to fall back on observation and inference from the buoy's movement at sea.

In low winds I examined the drogue underwater. It hung down vertically from the buoy and did not move away from small fragments of paper which I placed near it. In high winds direct underwater observation was not possible but the buoy's track was often so far away from downwind as to make it seem unlikely that it was carrying much leeway. Attention is drawn to the record of 19 June, where a westerly wind of force six blew for more than 6 h. The buoy ran 51° to the right of downwind and parallel to the coast. Unless leeway was being cancelled by a large onshore current, already seen to be very unlikely, this track represents true water movement. Even in a strong breeze, then, leeway is negligible.

My acknowledgements are due to the Tyne Improvement Commissioners for access to tide gauge information at South Shields, to the Coast Guards of Tynemouth for providing wind records and to Dr H. O. Bull and Dr J. N. Carruthers for valued advice. Especially is my gratitude due to R. Harrison, skipper of the R.V. *Alexander Meek* of the Dove Marine Laboratory, for the many patient hours he spent at sea with me in all weathers on this work.

SUMMARY

Observations have been made of the surface currents off the Northumberland coast over a period of 12 months, using a free-floating buoy. A mean vector current was found, setting 114° at 0·1 knot. Currents flowed into the quadrant to the right of downwind except when this would have directed them ashore. A relationship is established between wind speed and current speed.

REFERENCES

FULTON, T. W., 1897. The currents of the North Sea, and their relation to fisheries. *Rep. Fish. Bd Scot.*, 15th Year (1896), Pt. III, pp. 334–98.

HAY, R. F. M., 1954. A verification of Ekman's theory relating wind and ocean current directions using ocean weather ships' data. *Mar. Obs.*, Vol. 24, pp. 226–30.

LAWFORD, A. L. & VELEY, V. F. C., 1955. The relationship between local wind and current in coastal waters of the British Isles. *National Institute of Oceanography, Internal Report*, No. A3.

MEEK, A., 1926. The currents of the East Coast. *Rep. Dove mar. Lab.*, Vol. 15, pp. 38–42.

PROUDMAN, J., 1953. *Dynamical Oceanography*. London: Methuen.

TAIT, J. B., 1930. The surface water drift in the northern and middle areas of the North Sea and in the Faroe-Shetland channel. Part 1. *Sci. Invest., Fish. Bd Scot.*, 1930, No. 2, 82 pp.

—— 1937. The surface water drift in the northern and middle areas of the North Sea and in the Faroe-Shetland channel. Part 2. Section 3. *Sci. Invest., Fish. Bd Scot.*, 1937, No. 1.

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VITAMIN A AND CAROTENOIDS IN CERTAIN INVERTEBRATES

VI. CRUSTACEA: PENAEIDEA

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Fisher (1957) has pointed out the possible connexion between the richness in vitamin A and the pelagic existence of species of the orders Amphipoda and Mysidacea of the malacostracan Crustacea. The most striking example is seen in the Eucarida, of which the more benthic Decapoda are nearly all poorer in vitamin A than the pelagic Euphausiacea. In the Decapoda, species of the suborder Natantia do, however, lead a more or less pelagic life. Of its two constituent groups the Penaeidea swim more actively than the Caridea. The Penaeidea were believed by Calman (1910) to be primitive decapods and similarities in larval development indicated a possible affinity with the Euphausiacea. Gordon (1955) has recently produced further evidence of this relationship, particularly between euphausiids and sergestids, from the structure of the petasma, spermatophores, thelycum and photophores in the two groups. Taxonomically as well as ecologically, then, the penaeids may be said to lie between the rest of the decapods and the euphausiids, and we wished to compare these groups in their biochemical relationships so far as vitamin A and carotenoids were concerned. Moreover, some species of penaeids are the basis of important fisheries and knowledge of their vitamin A content might be of some economic value.

Published work on vitamin A and carotenoids in penaeids is confined almost entirely to that of Grangaud and his colleagues at Algiers on *Aristeomorpha foliacea* and *Aristeus antennatus*. These workers reported in several papers, summarized by Grangaud (1951) and Grangaud & Massonet (1951), on the anti-xerophthalmic activity of astaxanthin isomers from these species. Grangaud, Massonet & Sansac (1954) also mentioned the presence of vitamin A in concentrations of 5–10 i.u./g in the eyes and ovaries and of 30 i.u./g in the intestines of *Aristeomorpha foliacea*, *Aristeus antennatus* and *Parapenaeus longirostris*. In an earlier paper we reported vitamin A at a concentration of 5·7 i.u./g in the eyes of *Trachypenaeus membranaceus* from Naples (Fisher, Kon & Thompson, 1953).

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MATERIAL AND METHODS

Apart from *Trachypenaeus membranaceus* just mentioned we have analysed samples of thirteen species of penaeids, of which seven belong to the family Penaeidae and six to the Sergestidae. Details of these samples are given in Table 1.

Carotenoids and vitamin A were measured by the recent modification (Fisher, Kon & Thompson, 1956) of our usual method (Fisher, Kon & Thompson, 1952). None of the groups analysed contained enough specimens for biological assay or for the separation of vitamin A isomers carried out for some other Eucarida (Fisher, Kon & Plack, 1957; Wald & Brown, 1956–7; Wald & Burg, 1956–7).

RESULTS

Detailed results for the Penaeidae are shown in Table 2 and those for the Sergestidae in Table 3.

DISCUSSION

The results in the tables show that, apart from *Gennadas borealis*, all the species analysed contained vitamin A in at least one sample. The concentration of the vitamin in the Penaeidae was relatively low and its distribution varied. In some species it was confined to the eyes, in others to the bodies, and only in *Parapenaeopsis atlantica* was it found in both eyes and bodies. In the Sergestidae, vitamin A concentrations in most species tended to be higher than in the Penaeidae, but distribution varied in the different species.

All species of both families contained appreciable quantities of carotenoids and some, for example, *Petalidium foliaceum*, were very rich in them. The more actively pelagic sergestids usually contained only astaxanthin or its esters with an occasional trace of xanthophylls. There was a greater variety of carotenoids in the penaeids. Fisher, Kon & Thompson (1954) reported a similar difference between decapods with several different carotenoids and the more pelagic euphausiids in which only astaxanthin or its derivatives were found.

The results show that the concentration of vitamin A in penaeids is similar to those in other Natantia. Fisher (1957) has shown that, of the species we have examined, more Penaeidea (13 out of 14 listed in his paper) contain vitamin A than Caridea (29 out of 36). The results now presented indicate that Sergestidae with a more oceanic existence may be richer in vitamin A than Penaeidae, but more individuals of more species must be analysed in order to establish any such relationship.

Taken as a whole the vitamin A values for the Sergestidae would place them between the other Penaeidea (and other Decapods) and the Euphausiacea, a biochemical classification in agreement with current taxonomical views. *Penaeus aztecus*, the basis of an important shrimp fishery in Texas, contained modest amounts of vitamin A, all in the eyes and so of no importance in human nutrition.

TABLE 1. SPECIES, NUMBERS, SOURCES AND METHODS OF PRESERVATION OF PENAEIDS ANALYSED

Sample no.	Species	No. of specimens per sample	Locality	Ship	Date	How caught	Method of preservation
1	*Amalopenaeus elegans* Smith	16	39° 39′ N., 12° 08′ W.	*Discovery II*	16. xi. 1954	2 m stramin net	BF
2	*A. elegans* Smith	4	Bay of Biscay	*Sarsia*	18–24. vii. 1955	2 m stramin net	BF
3	*Funchalia woodwardi* Johnson	3	Madeira	—	17. v. 1955	From stomach of sword-fish	DA
4	*Gennadas borealis* Rathbun	7	32° 45′ N., 117° 38′ W.	*Paolina T*	4. iv. 1956	Isaacs-Kidd mid-water trawl	BF
5	*G. parvus* Bate	4	Bay of Biscay	*Sarsia*	28–30. iv. 1955	2 m stramin net	BF
6	*G. parvus* Bate	27	North Atlantic	*Discovery II*	vi. 1955	2 m stramin net	BF
7	*G. parvus* Bate	107	Bay of Biscay	*Sarsia*	18–24. vii. 1955	2 m stramin net	BF
8	*Parapenaeopsis atlantica* Balss	25	Gold Coast	—	Spring 1955	—	DS
9	*Penaeus aztecus* Ives	44	Gulf of Mexico	—	24. vi. 1952	—	DA
10	*Plesiopenaeus edwardsianus* (Johnson)	1	Madeira	—	1. vi. 1955	Deep fishing line	DA
11	*Petalidium foliaceum* Bate	29	32° 45′ N., 117° 38′ W.	*Paolina T*	4. iv. 1956	Isaacs-Kidd mid-water trawl	BF
12	*Sergestes arcticus* Kröyer	4	North Atlantic	*Discovery II*	16. xi. 1954	2 m stramin net	BF
13	*S. arcticus* Kröyer	4	North Atlantic	*Discovery II*	vi. 1955	2 m stramin net	BF
14	*S. arcticus* Kröyer	1	58° 54′ N., 13° 44′ W.	*George Bligh*	20. vii. 1955	Prawn trawl	BF
15	*S. arcticus* Kröyer	289	Bay of Biscay	*Sarsia*	18–24. vii. 1955	2 m stramin net	BF
16	*S. atlanticus* Milne Edwards	2	North Atlantic	*Discovery II*	16. xi. 1954	2 m stramin net	DA
17	*S. atlanticus* Milne Edwards	12	North Atlantic	*Discovery II*	vi. 1955	2 m stramin net	DA
18	*S. bisulcatus* Wood-Mason	6	32° 45′ N., 117° 38′ W.	*Paolina T*	4. iv. 1956	Isaacs-Kidd mid-water trawl	BF
19	*S. robustus* Smith	6	North Atlantic	*Discovery II*	16. xi. 1954	2 m stramin net	BF
20	*S. robustus* Smith	12	Bay of Biscay	*Sarsia*	18–24. vii. 1955	2 m stramin net	BF
21	*S. similis* Hansen	15	North Pacific	*Horizon*	26. v. 1953	1 m silk net	DS
22	*S. similis* Hansen	25	32° 45′ N., 117° 38′ W.	*Paolina T*	4. iv. 1956	Isaacs-Kidd mid-water trawl	BF

BF: boiled (Fisher, Kon & Thompson, 1952) and kept frozen until arrival at Shinfield laboratory.

DA: eyes dissected off and both parts preserved separately in alcohol; kept at low temperature, sent by air to London and immediately taken to Shinfield.

DS: dissected as above and preserved in alcohol, but sent by sea to England. *Parapenaeopsis atlantica* was stored in ship's refrigerator but *Sergestes similis* came by ordinary surface mail.

TABLE 2. OIL, VITAMIN A AND CAROTENOIDS IN SOME PENAEIDAE

Sample no.	Species	Av. wt. (mg)	Oil (%)	Vitamin A				Total carotenoids		β-carotene (µg/g)	Other carotenoids
				µg/spec.	µg/g	% ester	% alcohol	µg/spec.	µg/g		
1	*Amalopenaeus elegans*										
	Eyes (pairs)	0·14	9·1	0	0	0	0	0·28	2000	0	A
	Bodies	66	4·1	0·054	0·83	100	0	17	260	0	AE
	Total	66	4·1	0·054	0·82	100	0	17	270	0	AE
2	*A. elegans*										
	Eyes (pairs)	0·10	—	0	0	0	0	0·075	750	0	A
	Bodies	74	0·34	0	0	0	0	3·7	50	0	A
	Total	74	0·34	0	0	0	0	3·8	50	0	A
3	*Funchalia woodwardi*										
	Eyes (pairs)	240	3·1	0	0	0	0	2·8	12	0	A
	Bodies	17500	10	1·7	0·098	100	0	506	29	0·19	AE, C
	Total	17800	11	1·7	0·097	100	0	509	29	0·19	AE, C
4	*Gennadas borealis*										
	Eyes (pairs)	0·40	11	0	0	0	0	1·4	3500	0	A
	Bodies	350	12	0	0	0	0	9·0	26	0	AE
	Total	350	12	0	0	0	0	10	30	0	AE
5, 6	*G. parvus*										
	Eyes (pairs)	0·13	19	0·012	88	0	100	0·38	2900	0	AE
	Bodies	69	3·2	0	0	0	0	3·9	57	0	AE, X
	Total	69	3·3	0·012	0·17	0	100	4·3	62	0	AE, X
7	*G. parvus*										
	Whole specimens	12	4·6	0	0	0	0	2·3	190	0	AE, X
8	*Parapenaeopsis atlantica*										
	Eyes (pairs)	57	2·9	0·11	1·9	100	0	0·53	9·3	0	AE
	Bodies	6100	0·73	0·19	0·032	0	100	93	15	0·086	AE, C, X
	Total	6100	0·77	0·30	0·049	37	63	94	15	0·085	AE, C, X
9	*Penaeus aztecus*										
	Eyes (pairs)	51	11	0·22	4·3	67	33	0·96	19	0	A
	Bodies	4000	0·66	0	0	0	0	56	14	0·013	AE, C, X
	Total	4000	0·79	0·22	0·054	67	33	57	14	0·013	AE, C, X
10	*Plesiopenaeus edwardsianus*										
	Eyes (pairs)	470	3·5	0·69	1·5	100	0	50	150	0	AE
	Body	138000	10	0	0	0	0	19000	140	Trace	AE, C, X
	Total	138500	10	0·69	0·0050	100	0	19000	140	Trace	AE, C, X
—	*Trachypenaeus membranaceus**										
	Eyes (pairs)	100	3·0	0·23	2·2	—	—	0·54	5·3	0	A
	Bodies	8700	—	—	—	—	—	—	—	—	
	Total	8800	—	—	—	—	—	—	—	—	

* Result previously reported by Fisher *et al.* (1953) for comparison.

A = astaxanthin; AE = astaxanthin or its esters; C = carotene; X = xanthopyll.

TABLE 5. OIL, VITAMIN A AND CAROTENOIDS IN SOME SERGESTIDAE

Sample no.	Species	Av. wt. (mg)	Oil (%)	Vitamin A				Total carotenoids		Carotenoids present
				μg/spec.	μg/g	% ester	% alcohol	μg/spec.	μg/g	
11	*Petalidium foliaceum*									
	Eyes (pairs)	0·57	27	0	0	0	0	0·51	900	AE
	Bodies	180	14	0·37	2·0	10	90	60	330	AE, X
	Total	180	14	0·37	2·0	10	90	60	330	AE, X
12	*Sergestes arcticus*									
	Eyes (pairs)	0·88	2·9	0	0	0	0	5·0	5700	AE
	Bodies	170	2·2	0	0	0	0	11	65	AE
	Total	170	2·2	0	0	0	0	16	94	AE
13	*S. arcticus*									
	Eyes (pairs)	0·40	—	0	0	0	0	0·17	420	A
	Bodies	31	3·2	0	0	0	0	1·8	58	AE
	Total	31	3·2	0	0	0	0	2·0	63	AE
14	*S. arcticus*									
	Eyes (pairs)	1·0	30	0	0	0	0	0	0	—
	Body	23	17	0	0	0	0	3·0	130	AE
	Total	24	18	0	0	0	0	3·0	120	AE
15	*S. arcticus*									
	Eyes (pairs)	0·65	12	0·0065	10	50	50	0·0048	7·4	A
	Bodies	99	2·1	0·024	0·24	25	75	3·7	37	AE, X
	Total	100	2·1	0·031	0·31	32	68	3·7	37	AE, X
16	*S. atlanticus*									
	Eyes (pairs)	1·3	—	0·24	184	100	0	1·1	820	A
	Bodies	320	3·4	0	0	0	0	47	140	A
	Total	320	3·4	0·24	0·74	100	0	48	150	A
17	*S. atlanticus*									
	Eyes (pairs)	0·33	—	0·078	238	100	0	0·12	360	A
	Bodies	91	1·3	0	0	0	0	4·1	44	AE
	Total	91	1·3	0·078	0·85	100	0	4·2	46	AE
18	*S. bisulcatus*									
	Eyes (pairs)	6·3	9·5	0	0	0	0	1·4	220	A
	Bodies	510	5·1	3·4	2·3	80	20	127	84	AE, X
	Total	520	5·2	3·4	2·2	80	20	128	84	AE, X
19	*S. robustus*									
	Eyes (pairs)	0·37	4·5	0	0	0	0	0·63	1700	AE
	Bodies	74	0·22	0	0	0	0	11	140	AE
	Total	74	0·25	0	0	0	0	11	150	AE
20	*S. robustus*									
	Eyes (pairs)	0·84	—	0	0	0	0	0	0	—
	Bodies	230	6·0	0·092	0·40	67	33	5·7	25	AE, X
	Total	230	6·0	0·092	0·40	67	33	5·7	25	AE, X
21	*S. similis*									
	Whole specimens	52	2·9	0	0	0	0	0·65	12	AE
22	*S. similis*									
	Eyes (pairs)	2·7	—	0·076	28	63	37	0	0	—
	Bodies	210	3·1	0·55	2·6	83	17	20	97	AE, X
	Total	210	3·1	0·63	3·0	80	20	20	96	AE, X

β-carotene absent from all groups. A = astaxanthin; AE = astaxanthin or its esters; X = xanthophyll.

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SUMMARY

Vitamin A and carotenoids were measured in six species of Penaeidae and seven species of Sergestidae.

Vitamin A was present in at least one sample of all species except *Gennadas borealis* at concentrations mostly of the same order as previously found in other Decapoda, but usually higher in the Sergestidae than in the Penaeidae.

Carotenoids in the Penaeidae included astaxanthin and its esters, carotenes and xanthophylls, but only astaxanthin or its esters with occasional traces of xanthophylls were found in the Sergestidae.

REFERENCES

Calman, W. T., 1910. *Oxford Treatise on Zoology, Part VII. Appendiculata. Third Fascicle. Crustacea.* London: Black.

Fisher, L. R., 1957. 'Vitamins'. In *The Physiology of Crustacea* (ed. T. H. Waterman). New York: Academic Press (*in the Press*).

Fisher, L. R., Kon, S. K. & Plack, P. A., 1957. Vitamin A isomers in some eucaridan Crustacea. *Proc. roy. Soc.* B, Vol. 147, pp. 200–17.

Fisher, L. R., Kon, S. K. & Thompson, S. Y., 1952. Vitamin A and carotenoids in certain invertebrates. I. Marine Crustacea. *J. mar. biol. Ass. U.K.*, Vol. 31, pp. 229–58.

—— —— —— 1953. Vitamin A and carotenoids in some Mediterranean Crustacea: with a note on the swarming of *Meganyctiphanes*. *Bull. Inst. océanogr. Monaco*, No. 1021, 19 pp.

—— —— —— 1954. Vitamin A and carotenoids in certain invertebrates. II. Studies of seasonal variations in some marine Crustacea. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 589–612.

—— —— —— 1956. Vitamin A and carotenoids in certain invertebrates. IV. Mollusca: Loricata, Lamellibranchiata, and Gastropoda. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 41–61.

Gordon, I., 1955. Importance of larval characters in classification. *Nature, Lond.*, Vol. 176, pp. 911–12.

GRANGAUD, R., 1951. L'astaxanthine nouveau facteur vitaminique A. *Actualités biochim.*, No. 15, 49 pp.

GRANGAUD, R. & MASSONET, R., 1951. Activité vitaminique A de l'astaxanthine. *Tunis. méd.*, T. 29, pp. 680–3.

GRANGAUD, R., MASSONET, R. & SANSAC, A., 1954. Activité antixérophtalmique du pigment caroténoïde de Pénéidés (*Aristeomorpha foliacea* Risso et *Aristeus antennatus* Risso). Technique de préparation des extraits actifs. *C.R. Soc. Biol., Paris*, T. 148, pp. 533–6.

WALD, G. & BROWN, P. K., 1956–7. The vitamin A of a euphausiid crustacean. *J. gen. Physiol.*, Vol. 40, pp. 627–34.

WALD, G. & BURG, S. P., 1956–7. The vitamin A of the lobster. *J. gen. Physiol.*, Vol. 40, pp. 609–25.

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THE IRON CONTENT OF SEA WATER

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Concentrations of iron in sea water up to about 3 mg Fe/l. have been reported, most of the figures being in the range 10–100 μg Fe/l. The published analyses, with notes on the methods used where these are known, are summarized by Lewis & Goldberg (1954), who list thirty-nine sources up to 1953.

It is well known that much of the iron is in particles which can be removed by filtration. Attempts have been made to estimate the chemically more reactive and presumably biologically more available fraction of the metal. Often the coarser particles have been filtered off before analysis, and reagents of varying potency have been used to bring iron into solution for colorimetric determination. These procedures have sometimes excluded determination of the total concentration of iron in the water. It is known, however, that the phytoplankton can utilize particulate material for its requirements of iron (Allen & Nelson, 1910; Harvey, 1927; Goldberg, 1952).

Methods intended to determine total iron in sea water have been described by Thompson, Bremner & Jamieson (1932); Thompson & Bremner (1935); Cooper (1935, 1948); Rakestraw, Mahncke & Beach (1936); and Lewis & Goldberg (1954). Thompson and his collaborators evaporated the water with excess of sulphuric acid and heated to fuming, and Lewis & Goldberg used perchloric acid in a similar way. These methods should undoubtedly be effective, as should that used by Rakestraw *et al.* when applied to unfiltered water. Cooper, in his method for 'total iron' (for which expression he made specific reservations) used a less drastic attack and heated the sample with hydrochloric acid (0·008 N) and some bromine, excess of bromine being later boiled off. Some analyses of suspended matter in sea water (Armstrong & Atkins, 1950) showed that in surface water from a position in the English Channel there were present from 42 to 210 μg Fe/l. These amounts were so much greater than those previously found at the same position, by direct analysis, that it seemed desirable to make more analyses and to re-examine the analytical methods.

ANALYTICAL CONSIDERATIONS

Methods using wet ashing with sulphuric or perchloric acids should serve well for referee analyses. They are not, however, very convenient for routine use and reagent blanks tend to be high. A simple alternative way of bringing iron into solution which could be used for large batches of samples was sought. A possible method came from the observation that when the suspended matter is filtered from sea water, the iron can be extracted completely from the solid residue with cold 1 N hydrochloric acid. This concentration is unmanageably great for acidification of a sea water sample of about 100 ml., so attempts were made to use 0·1 N acid. At this level blanks are low and partial neutralization is easier. 0·1 N acid was ineffective in the cold, although boiling for 1 h dissolved more than 90% of the iron present. Solution of iron appeared to be complete however when the acidified sample was heated in an autoclave for 5 h at 140° C. Since the treatment extracted appreciable and varying amounts of iron from borosilicate glassware, fused silica flasks had to be used. It proved convenient to add 1 ml. of concentrated hydrochloric acid to 85 ml. of sea water to give a concentration of 0·12–0·14 N. After digestion and adjustment of volume the addition of 10 ml. (20%, w/v) sodium acetate brought the solution to pH 3·8 ± 0·2, which was suitable for colorimetric determination.

Of the many very satisfactory colorimetric reagents for iron, 1:10 phenanthroline seemed to be the most suitable. It was extensively tested by Fortune & Mellon (1938), who found it effective (with ferrous iron) in the pH range 2–9. Of fifty-five ions which these authors tested for interference none of the undesirable ones, with the possible exception of fluoride, is present in sea water in troublesome quantity. 2-2′-Dipyridyl, of similar sensitivity and also interference-free, has a narrower pH range of 3·5–8·5 (Hill, 1930). It could be used, but extra sodium acetate might be desirable to raise the pH somewhat (since colour development tends to be slow at the higher acidities) and this would undoubtedly increase blanks.

Some tests with 1:10 phenanthroline were made to see whether fluoride would interfere with iron determinations at pH 3·8, and it was found that there was no effect up to a concentration of 7·6 mg F/l. (400 μg atom F/l.). This is 5 or 6 times the usual concentration in sea water, which is stated to be about 1·4 mg F/l. (74 μg atom F/l.) (Thompson & Taylor, 1933).

It was found that 1:10 phenanthroline could be used with confidence and that the method prescribed by Fortune & Mellon, using hydroxylamine hydrochloride for reduction of ferric iron, could be followed closely with sea water. The depth of colour, which does not fade, is unaffected by the salts in the water, and Beer's Law is obeyed in a simple filter absorptiometer.

METHOD

Apparatus

Polyethylene sample bottles. Fused silica flasks, 100 ml. Autoclave for temperature 140° C (40 p.s.i. or 2·8 kg/cm^2). This should be of non-ferrous construction. Absorptiometer to take 10 cm cuvettes or longer. Glassware should be cleaned with strong hydrochloric acid before use and reserved for this work.

Reagents

Standard iron solution. 1 ml. ≡ 0·0001 g Fe. Dissolve 0·702 g $FeSO_4.(NH_4)_2SO_4.6H_2O$ in 1%, v/v, HCl and make to 1 l. with 1%, v/v, acid.

Sodium acetate 20%, *w/v*. Dissolve 200 g $CH_3COONa.3H_2O$ in water, make to 1 l., shake with about 10 ml. chloroform to saturate and filter on No. 42 Whatman paper.

Hydroxylamine hydrochloride 10%, *w/v*. Dissolve 10 g $NH_2OH.HCl$ in water and make to 100 ml.

1:10 *phenanthroline* 0·1%, *w/v*. Dissolve 0·25 g 1:10 phenanthroline in hot water, cool and make to 250 ml.

Analysis of sea water

Measure 85 ml. of the properly collected (see below) and well-shaken sample into a 100 ml. silica flask and add 1·0 ml. conc. HCl, preferably with a syringe pipette. Cover the flask and heat for 5 h in an autoclave at 140° C. After cooling, adjust the volume to 86 ml., transfer to a 150 or 250 ml. borosilicate flask and add 10 ml. 20% sodium acetate and 1 ml. 10% $NH_2OH.HCl$, using pipettes. Mix the solution, and measure the absorbance in a 10 or 15 cm cuvette at about 510 mμ (Ilford no. 603 or combination of 303 and 404 filters). This reading will allow correction for slight turbidity of the solution. Return the solution from the cuvette to the flask, add 3 ml. 0·1% 1:10 phenanthroline solution, mix, and measure the absorbance again after 10 min. The difference between the two readings, when corrected for the absorbance of the blank, measures the iron content of the sample.

Blank determination

Carry 85 ml. of iron-free distilled water through the same procedure. If it is found that release of iron from the flasks is negligible digestion of blanks may of course be omitted.

Calibration

In a series of 100 ml. graduated flasks, place measured amounts of the standard iron solution (or of a freshly prepared known dilution of it), to give a range of known iron concentrations. They may be chosen on the assumption that about 60μg Fe in 100 ml. are required to give an absorbance of 1·0 in a 10 cm cuvette in a filter absorptiometer at about 510 mμ. Add distilled water to a volume of about 80 ml., and then add HCl, sodium acetate, $NH_2OH.HCl$ and 1:10 phenanthroline in the quantities given above. Adjust the volumes to 100 ml., mix and measure absorbance after 10 min. Construct a calibration curve. This should be a straight line. If so, it is convenient to recalculate the slope as the concentration of iron in an 85 ml. sea-water sample required to give an absorbance of 1·00. The product of this factor and the corrected absorbance of a treated sea-water sample is the concentration of iron in the sample. Calibration should be repeated occasionally as a check on the constancy of the absorptiometer. If the curve is linear a check at one iron concentration is enough.

TESTS OF METHOD

The effectiveness of the digestion in extracting iron from refractory marine material was tested by comparing the amount brought into solution by digestion with that found after ignition and fusion with potassium bisulphate. For this test two samples, one of marine silt and the other of equal parts of dried whole fish meal and dried ground sea weed, were homogenized by grinding. Fibrous material in the second sample was removed with a 100-mesh sieve. Portions of 50–100 mg were weighed and assayed by the two methods. The results, expressed as iron contents of the samples, are given in Table 1, and show that recovery of iron by the digestion method was sensibly complete.

For trials with sea water, two carboys of freshly collected water were well shaken and kept vigorously stirred whilst samples were drawn off by siphon. From each carboy three samples of 2 l. each were taken for filtration as described below, and three sets of eight samples for replicate determinations by the digestion method (85 ml.), Thompson & Bremner's sulphuric acid method (100 ml.) and Cooper's 'Total Iron' method (150 ml.). The 2 l. samples were filtered on 'Gradocol' membrane filters (A.P.D. approx. 1 μ) and the filtrates reserved. Each membrane with the suspended matter on it was ignited in platinum and the residue fused with bisulphate. Iron in the melt was determined, blanks being carried through all stages. To the iron concentrations thus found were added those found in the filtrates by the digestion method. Any material passing the filter was necessarily very finely subdivided and was assumed to be readily dissolved. (The amount of iron

TABLE 1. RECOVERY OF IRON FROM MARINE MATERIALS

	Iron content (%)							
	Ignition and bisulphate fusion				Acid digestion at 140° C			
Material								
Silt	1·26	1·28	1·26	1·25	1·24	1·27	1·25	1·27
Sea weed and fish meal	0·061	0·077	0·060	0·069	0·079	0·078	0·067	0·060

TABLE 2. COMPARISON OF METHODS FOR DETERMINATION OF TOTAL IRON IN SEA WATER

(Iron found, μg Fe/l.)

	Filtration, bisulphate fusion, etc.	Acid digestion at 140° C	Fuming with H_2SO_4 (Thompson *et al.*)	HCl and Br_2 (Cooper)
Carboy A	89	81	71	13
	100	78	100	15
	83	83	98	21
	—	80	83	15
	—	82	83	17
	—	89	79	18
	—	93	76	19
	—	95	84	13
Mean	91	85	84	16
S.D.		±6	±9	±3
Carboy B	140	106	130	50
	164	152	132	34
	118	139	168	6
	—	143	137	9
	—	140	140	17
	—	145	137	21
	—	142	132	22
	—	139	132	21
Mean	141	138	138	23
S.D.		±14	±12	±14

found in these filtrates varied from 4 to 8 μg Fe/l., and may be compared with the 2–5·5 μ Fe/l. found after filtration through 'Millipore' membranes of 0·5 μ A.P.D. by Lewis & Goldberg.) This procedure gave an independent estimate of total iron in the samples, although it is probably not very accurate because so much manipulation is involved.

The samples for digestion were carried through the method described above. Those for test by Thompson & Bremner's method were heated to fuming as prescribed in the original method. Silica flasks were used. The colorimetric finish with thiocyanate, however, was set aside in favour of the 1:10 phenanthroline procedure after neutralization of excess acid with ammonia, iron in this reagent being allowed for. The samples for Cooper's 'Total Iron' method were treated as described in his 1948 paper, dipyridyl being used.

The results are given in Table 2. Taking into consideration the marked scatter in the figures it can be seen that the filtration, Thompson & Bremner's and the new digestion methods agree well, but that the HCl-bromine 'Total Iron' method is not rigorous enough, as Cooper himself surmised (1948, p. 281).

COLLECTION OF SAMPLES

Circumspection is obviously needed when samples are taken from a steel ship and in a hydrographic water bottle on a steel wire. Ordinary glass sample-bottles are unsuitable, even after washing with acid, since they are appreciably attacked by sea water. Iron in the glass is released, remaining on the walls of the bottle, probably as a film of ferric hydroxide, but easily contaminating the sample. Moreover, Goldberg (1952) showed that iron added to sea water is rapidly adsorbed by glass. Polyethylene bottles, though not ideal, may be used. Analysis of some new bottles showed the plastic to be iron-free, but it should be remembered that the bottles may be made on iron or steel moulds. It is advisable to wash out all bottles with strong hydrochloric acid before use (a little wetting agent such as cetyl ammonium bromide with the acid is helpful) and to test for extractable iron by filling with 0·1 N-HCl and heating for several hours in a water bath at 100° C and then determining iron in the solution. It has been noticed that polyethylene bottles which had been used repeatedly for collection of water for other analyses had an internal deposit of ferruginous material which came out only after prolonged acid treatment.

Deposition of iron on the walls of polyethylene bottles takes place rapidly from raw sea water. Experiments showed that in a week one-quarter of the iron originally present was so deposited; storage for 4 weeks showed losses of one-half to two-thirds. The iron deposited is difficult to recover, only half of it being removed by standing with 0·1 N-HCl for several days with occasional shaking. It is advisable therefore to complete iron determinations soon after collection. When this cannot be done the samples should be acidified when collected. For long storage it would probably be advisable to use the full amount of acid, i.e. 1·0 ml. conc. HCl per 85 ml. of sample, but for 3 weeks' storage a smaller amount is sufficient. Table 3 shows the effectiveness of addition of 1 ml. of 10%, v/v, HCl per 100 ml. sample. This amount of acid may be disregarded when acidifying before digestion and its contribution to the reagent blank is usually negligible.

TABLE 3. IRON DETERMINATIONS ON SUBSAMPLES OF SEA WATER BEFORE AND AFTER STORAGE FOR 3 WEEKS WITH ADDITION OF 1 ML. (10%, V/V) HCl PER 100 ML.

Before storage	74, 94, 92, 81, 105, 86, 85, 88, 67, 88	Mean 86	S.D. ± 11 µg Fe/l.
After 3 weeks' storage	105, 83, 86, 86, 80, 81, 86, 88, 88, 84	Mean 86	S.D. ± 7 µg Fe/l.

VARIABILITY OF RESULTS

Tables 2 and 3 include standard deviations. The sets of samples analysed are hardly random ones, but subsamples of larger portions which were vigorously stirred whilst subsampling. Those of Table 2 were, as stated, from carboys (20–25 l.), whilst those of Table 3 were from a bucket (8 l.). A set of ten samples, each of 85 ml., from ten consecutive buckets of water taken from the sea surface at Station E 1 whilst the ship was stopped gave a mean iron content of 65 ± 22 µg Fe/l. Sampling took about 15 min.

The variability of replicate iron determinations has been discussed by other workers, and was turned to account by Cooper (1948) who assessed, by statistical treatment, the size and distribution of iron-containing particles in the water. Lewis & Goldberg (1954) were at some pains to obtain replicate samples from deep water and gave a statistical analysis of their data from nine Pacific Ocean stations.

RESULTS OBTAINED

Iron concentrations found by the digestion method in samples from the Plymouth Laboratory stations L2 to L6 and at the International Hydrographic Station E1 during 1955 and 1956 are given in Table 4. As would be expected there is appreciably more iron in the coastal waters. There is a seasonal variation, more iron being found in the winter months, which may be caused by the increased run-off from the land and greater turbulence in winter. There is often, at Station E 1, more iron at the surface, as observed by Cooper (1948). Iron concentrations are very much higher than those reported by Cooper for 1933 and 1934 and 1946 and 1947. This is ascribable to the difference in the analytical methods.

Results from two other stations in the English Channel and from three positions off the Brittany coast in the northern part of the Bay of Biscay are given in Table 5. Figures from four deep-water stations in the Bay of Biscay are given in Table 6.

TABLE 4. IRON CONTENT OF SEA-WATER SAMPLES FROM POSITIONS NEAR PLYMOUTH 1955–6

(In μg Fe/l.)

St. no.	N. lat.	W. long.	Miles from Plymouth	Depth (m)	1955											
					18 Jan.	16 Feb.	15 Mar.	12 Apr.	9 May	13 June	13 July	11 Aug.	15 Sept.	18 Oct.	17 Nov.	21 Dec.
L2	50° 20′	4° 10′	2	0	203	160	58	146	—	33	64	157	53	169	177	424
L3	50° 18′	4° 11′	5	0	82	174	59	65	—	21	17	92	35	176	129	212
L4	50° 15′	4° 13′	8	0	90	107	67	68	—	7	16	100	157	94	102	100
L5	50° 11′	4° 18′	12	0	92	92	43	111	—	8	11	140	115	85	77	59
L6	50° 06′	4° 21′	17	0	124	326	60	25	—	5	30	107	97	65	62	94
E1	50° 02′	4° 22′	22	0	78	111	39	89	13	13	1	58	75	25	127	100
				5	49	77	23	36	17	198	6	31	22	19	26	26
				10	60	61	27	15	19	19	4	24	29	33	22	38
				20	—	—	—	—	—	—	3	51	15	48	35	64
				25	85	77	31	17	15	7	—	—	—	—	—	—
				50	34	90	26	50	21	16	7	31	57	84	56	83
				70	73	108	29	84	46	25	4	60	28	175	54	87
Integral mean, Station E1				—	61	92	28	41	22	28	5	42	36	72	45	70

St. no.	Depth (m)	1956													
		17 Jan.	21 Feb.	26 Mar.	11 Apr.	16 Apr.	24 Apr.	22 May	23 July	22 Aug.	25 Sept.	23 Oct.	13 Nov.	10 Dec.	Mean
L2	0	239	129	209	106	103	54	69	40	84	70	104	120	86	127
L3	0	150	60	101	86	108	27	45	21	64	65	38	86	74	83
L4	0	121	60	33	129	98	19	32	73	57	37	30	42	64	71
L5	0	83	56	35	88	100	16	42	53	54	20	30	47	46	63
L6	0	76	64	31	52	50	28	38	75	76	13	44	64	78	70
E1	0	28	57	31	16	20	7	15	69	126	77	24	31	48	51
	5	36	36	10	9	13	5	50	14	134	20	8	32	31	37
	10	35	31	10	9	11	4	31	25	10	21	7	34	31	24
	20	37	26	20	11	13	8	13	21	15	18	10	43	23	27
	25	—	—	—	—	—	—	—	—	—	—	—	—	—	
	50	52	36	13	14	12	7	20	28	16	18	8	53	79	36
	70	75	32	18	20	22	19	16	33	25	17	13	59	41	46
Integral mean, Station E1	—	46	33	16	13	14	9	20	26	29	20	12	47	48	35

TABLE 5. IRON CONTENT OF SEA-WATER SAMPLES FROM POSITIONS IN ENGLISH CHANNEL AND OFF BRITTANY COAST

(Depth in metres. Iron content in µg Fe/l.)

49° 27′ N., 4° 42′ W. 14 Nov. 1956		48° 34′ N., 5° 13′ W. 14 Nov. 1956		48° 18′ N., 5° 18′ W. 14 Nov. 1956	
Depth	Iron	Depth	Iron	Depth	Iron
0·5	45	0	66	0	90
5	33	5	30	5	27
10	45	10	56	10	25
20	34	20	52	20	31
50	37	50	53	50	35
90	19	100	14	100	35
		110	35	105	41

47° 50′ N., 5° 22′ W. 14 Nov. 1956		47° 35′ N., 4° 20′ W. 15 Nov. 1956	
Depth	Iron	Depth	Iron
0	61	0	126
5	61	10	26
10	62	20	28
20	61	50	40
50	49	75	24
100	86	100	109
125	158	105	126

TABLE 6. IRON CONTENT OF SEA-WATER SAMPLES FROM POSITIONS IN THE BAY OF BISCAY

(Depth in metres. Iron content in µg Fe/l.)

48° 00′ N., 10° 05′ W. 29 Apr. 1955		47° 33′ N., 07° 27′ W. 8 May 1955		46° 27′ N., 08° 04′ W. 27 June 1955		47° 30′ N., 08° 00′ W. 28 May 1956	
Depth	Iron	Depth	Iron	Depth	Iron	Depth	Iron
0	46	10	17	0	23	90	21
10	13	100	154	10	7	170	6
100	45	200	290	50	11	260	7
600	10	265	22	100	7	350	9
745	10	275	7	200	146	490	4
885	13	355	17	300	15	660	4
1025	11	435	29	400	13	860	5
1280	7	600	27	500	21	1100	9
1670	11	720	22	585	8	1500	6
2060	8	810	50	655	26	1890	7
2650	1	910	39	780	10	2180	11
2950	21	1050	34	930	27	2580	9
3250	58	1190	77	1080	9	3190	4
3540	37			1150	9	3700	7
				1320	15	4000	8
				1520	9	4570	5
				1720	16		
				1945	5		
				2245	7		
				2540	6		
				2840	10		
				3140	13		
				3440	12		
				3700	5		
				3990	18		
				4270	9		
				4460	4		

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SUMMARY

A method, suitable for routine use, of determining total iron in sea water is described. The water is acidified to about 0·13 N with hydrochloric acid and is heated for 5 h in an autoclave at 140° C. Iron is determined absorptiometrically with 1:1 phenanthroline after reduction with hydroxylamine hydrochloride.

Tests of the method, collection and storage of samples and the variability of results are described.

Iron contents of water samples from the English Channel and the Bay of Biscay are reported.

REFERENCES

ALLEN, E. J. & NELSON, E. W., 1910. On the artificial culture of marine diatoms. *J. mar. biol. Ass. U.K.*, Vol. 8, pp. 421–74.

ARMSTRONG, F. A. J. & ATKINS, W. R. G., 1950. The suspended matter of sea water. *J. mar. biol. Ass. U.K.*, Vol. 29, pp. 139–43.

COOPER, L. H. N., 1935. Iron in the sea and in marine plankton. *Proc. roy. Soc.* B, Vol. 124, pp. 419–38.

—— 1948. The distribution of iron in the waters of the Western English Channel. *J. mar. biol. Ass. U.K.*, Vol. 27, pp. 279–313.

FORTUNE, W. B. & MELLON, M. G., 1938. The determination of iron with *O*-phenanthroline. *Industr. Engng Chem.* (*Anal.*), Vol. 10, pp. 60–4.

GOLDBERG, E. D., 1952. Iron assimilation by marine diatoms. *Biol. Bull., Woods Hole*, Vol. 102, pp. 243–8.

HARVEY, H. W., 1927. The supply of iron to diatoms. *J. mar. biol. Ass. U.K.*, Vol. 22, pp. 205–19.

HILL, R., 1930. A method for the estimation of iron in biological material. *Proc. roy. Soc.* B, Vol. 107, pp. 205–14.

LEWIS, G. J. & GOLDBERG, E. D., 1954. Iron in marine waters. *J. mar. Res.*, Vol. 13, pp. 183–97.

RAKESTRAW, N. W., MAHNCKE, H. E. & BEACH, E. F., 1936. Determination of iron in sea water. *Industr. Engng Chem.* (*Anal.*), Vol. 8, pp. 136–8.

THOMPSON, T. G. & BREMNER, R. W., 1935. The occurrence of iron in the waters of the north-east Pacific Ocean. *J. Cons. int. Explor. Mer*, Vol. 10, pp. 39–47.

THOMPSON, T. G., BREMNER, R. W. & JAMIESON, M., 1932. Occurrence and determination of iron in sea water. *Industr. Engng Chem.* (*Anal.*), Vol. 4, pp. 288–90.

THOMPSON, T. G. & TAYLOR, H. J., 1933. Determination and occurrence of fluorides in sea water. *Industr. Engng Chem.* (*Anal.*), Vol. 5, pp. 87–9.

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MARINE ALGAL ZONATION AND SUBSTRATUM IN BEER BAY, SOUTH-EAST DEVON

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(Text-figs. 1–4)

A preliminary examination of the marine algal zonation of Beer Bay, south-east Devon, indicated that there might be some divergences from the basic zonation on the south coast of England as described by Grubb (1936) and Evans (1947). Moreover, it appeared that the divergences might be due to an effect of the substratum being superimposed upon the normal intertidal zonation. To investigate this further a survey of the marine algal vegetation and the substratum of the intertidal regions of Beer Bay was carried out.

Beer is situated approximately at the centre of Lyme Bay (National Grid 30/2389). The Bay is shown in detail in Fig. 1, with the areas surveyed stippled. The Bay is exposed from the south-east to the south. Although the south-east gales can be severe at Beer, they are less frequent than gales from the south-west, which seem to produce the most severe wave-action in the Bay. In these gales, the waves, which are travelling in a north-easterly direction outside the headland, seem to alter their direction, and travelling due north, sweep right into the Bay and cause considerable wave-action, even along the cliffs on the west side. The exposed nature of the Bay is reflected in the absence of *Pelvetia canaliculata* Dcne. & Thur. and *Ascophyllum nodosum* Le Jol., the almost complete absence of *Fucus spiralis* L., and the occurrence of *Alaria esculenta* Grev. at Beer Head and The Hall (Fig. 1).

Throughout this survey the marine algae were identified from the *Handbook of the British Seaweeds* (Newton, 1931), and the names used are those given in Parke's (1953) Check List.

SUBSTRATUM

The most westerly outcrop of the Chalk on the south coast of England occurs at Beer, and as a result the cliffs have been described by several authors (Meyer, 1874; Rowe & Sherborne, 1903; Jukes-Browne, 1904). The cliffs surrounding the Bay consist almost entirely of the Upper, Middle and Lower Chalks, but there is a zone of 2·5–3 m at the base of the cliffs where the chalk passes into the Upper Greensand. Directly beneath the clearly defined Lower Chalk there is a 1 m bed of harder rock, which Rowe & Sherborne (1903) described as a transition bed between the Lower Chalk and Upper Greensand. They called this the Cenomanian Limestone. Jukes-Browne (1904), Dewey (1948), and Woodward & Ussher (1911) all include this bed in the Lower Chalk and apply the term Cenomanian to all the Lower Chalk at Beer. Below this zone occurs the siliceous rock shown as the Upper Greensand and it is the harder parts of this Greensand that form most of the flat expanse of rock which is exposed on each side of the Bay at low tide. However, in a few places, particularly on the east side of the Bay, the Cenomanian Limestone persists on top of the Greensand and takes the form of large flat-topped rocks projecting well above the general level of the surrounding Greensand. At the base of the cliffs there are areas of chalk boulders which are the remains of extensive falls of cliff. Although these are much softer than fragments of the Greensand or of the Limestone, and tend to be eroded away rather quickly, they are continually being replaced by minor cliff falls. The beach is entirely of pebbles which range in size from stones 10–15 cm in diameter down to a fine shingle. Pebbles also occur in small isolated areas along the foot of the western cliffs where there are no chalk boulders. In the centre of the Bay and below the lowest tides there is a rather coarse sand.

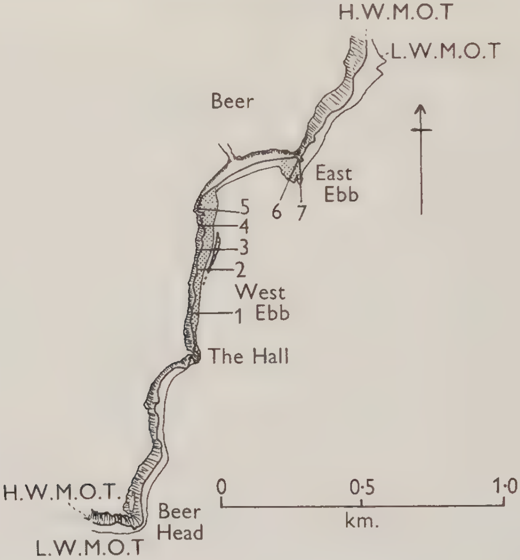

Fig. 1. Map of Beer Bay. Areas which were investigated are stippled. Numbered lines indicate positions of transects.

METHODS OF SURVEY

Plane-table maps

Plane-table maps of the two sides of the Bay were prepared on a scale of 30 ft. to 1 in. and various fixed points marked on them to facilitate later work. The rock outcrops on both sides of the Bay were only mapped down as far as extreme low-water spring tides. In August of both 1954 and 1955 the substratum and the zones of the dominant algae were plotted on these maps and they are reproduced on a reduced scale as Figs. 2 and 3.

Transects

In 1955, seven transects, down through the intertidal zone, were investigated. The positions of these transects are shown on Fig. 1. Levelling along these transects was done with a modified form of the levelling apparatus developed by Miss J. M. Kain (in preparation). On a calm day the height of the sea in relation to fixed points on the transects was recorded and with the aid of a tidal curve and the predicted range of the tide for that day, which were kindly supplied by the Hydrographer to the Admiralty, the mean tidal levels, as quoted by the Admiralty, were found for each transect. This was later checked by levelling back to a bench mark.

RESULTS OF THE SURVEY

Substratum maps

Greensand, limestone, chalk boulders and pebbles are plotted on the substratum maps, and the areas colonized by the reef-building worm *Sabellaria alveolata* are also indicated. These worms build up extensive colonies of honeycomb-like structure at certain points between high-water neap tides and low-water spring tides. The colonies are usually quite extensive and vary considerably in depth. In some places they form a uniform cover 2–5 cm deep and in other places they are in the form of tussocks. As will be shown later it is mainly on these colonies that the anomalies in the normal zonation occur.

Species maps

Five main groups are considered in the species maps, these are: *Enteromorpha* spp., *Ulva lactuca*, *Fucus vesiculosus*, *F. serratus* and *Gigartina stellata*. *Enteromorpha* was not divided up into species, but it was known to include *Enteromorpha intestinalis* and *E. compressa* and probably several other species. The *Fucus vesiculosus* was a form without vesicles and was probably the form described in Newton (1931) as *F. vesiculosus* var. *evesiculosus* Cotton. Throughout the area this was the only form of *F. vesiculosus* found, and Knight & Parke (1950) say: ‘An almost complete absence of vesicles characterizes the whole *F. vesiculosus* zone on the Devon, Manx and Argyll coast, in very exposed places such as at the foot of vertical cliffs exposed to full surf action.' At Beer this alga is not growing on vertical cliffs but, as already mentioned, there is considerable wave action in the Bay. *Laminaria* spp. were not plotted as these only became dominant below the level of the mapped areas. It should be recorded that, although in several places the high-tide mark was well up the cliffs, there were none of the zones described by Anand (1937) as being characteristic of the chalk cliffs of Sussex.

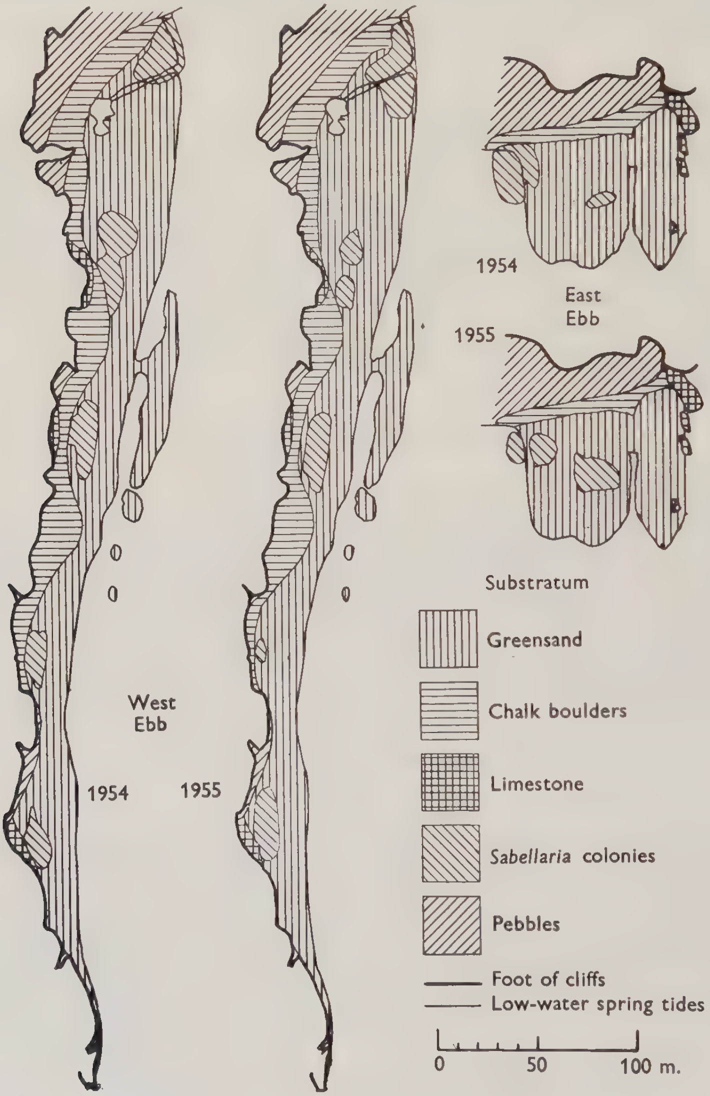
1954
East Ebb
1955
Substratum
Greensand
Chalk boulders
Limestone
Sabellaria colonies
Pebbles
Foot of cliffs
Low-water spring tides
0
50
100 m.
West Ebb
1954
1955

Fig. 2. Substratum maps 1954 and 1955.

Species and substratum

The maps of the west side of the Bay show five areas colonized by the *Sabellaria* worms and the major parts of these colonies were dominated by *Ulva lactuca* or *Gigartina stellata*. On the east side of the Bay the worm colonies were also dominated by either *Ulva* or *Gigartina*. It should, however, be pointed out that *Gigartina* was also dominant on some extensive areas that

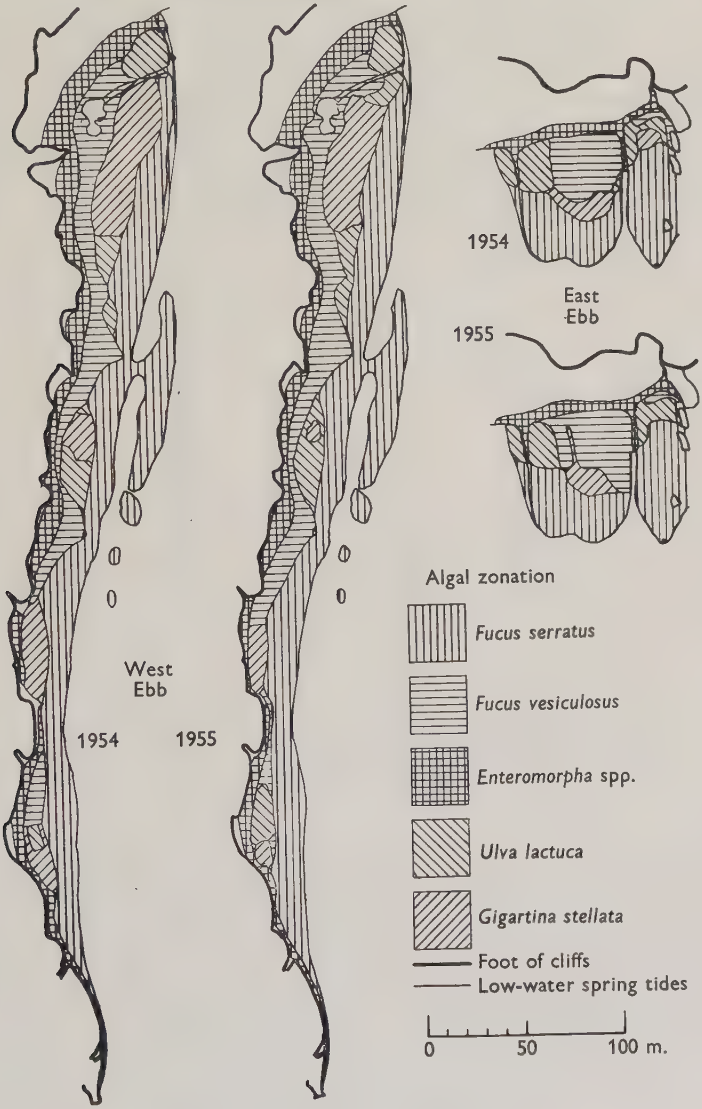
1954
East
Ebb
1955
Algal zonation
Fucus serratus
Fucus vesiculosus
Enteromorpha spp.
Ulva lactuca
Gigartina stellata
Foot of cliffs
Low-water spring tides
0
50
100 m.
West
Ebb
1954
1955

Fig. 3. Algal zonation 1954 and 1955.

were not colonized by the *Sabellaria* worms but, apart from one or two very small areas, *Ulva* was only dominant on areas colonized by the *Sabellaria* worms. The chalk boulders at the base of the cliffs were almost entirely covered with a dense growth of *Enteromorpha*. This growth was correlated with the occurrence of fresh water running out of the Greensand at the base of the cliffs and forming streamlets running between these chalk rocks. *Fucus vesiculosus* dominated most of the rest of the chalk rocks which did not extend down low enough to have much growth of *F. serratus*. In several places there was *F. serratus* around the base of these rocks but *F. vesiculosus* on the top and sides. Most of the Greensand was dominated by *F. serratus*, but there were areas where *Gigartina* was dominant, and, where the Greensand was exposed at high enough levels, *Fucus vesiculosus* was dominant. The pebbles were not stable enough for any macroscopic algal growth although, where they occurred along the foot of the western cliffs, they were often covered with diatoms.

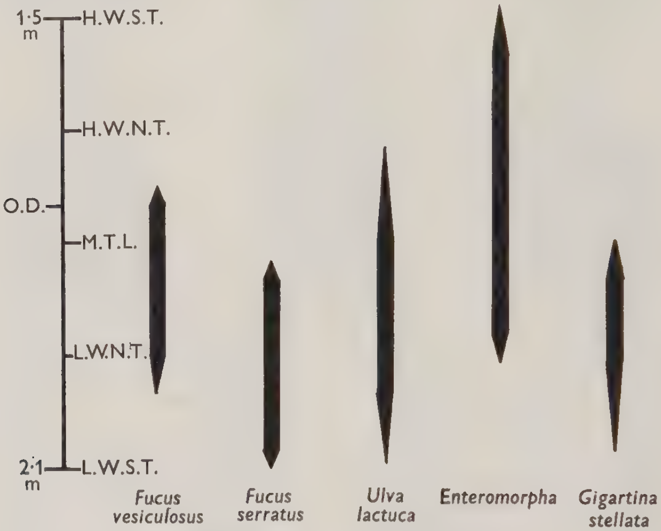

Fig. 4. Distribution diagrams for 1955.

Transects

From the transects distribution diagrams were prepared for *Enteromorpha* spp., *Ulva lactuca*, *Fucus vesiculosus*, *F. serratus* and *Gigartina stellata*, and these are shown in Fig. 4. When these diagrams were compared with those of Evans (1947) for the Plymouth region, it was found that in both areas the means of *Fucus vesiculosus* and *F. serratus* were approximately the same, but at Beer the zones of both species did not extend as far up, or as far down, as they did near Plymouth. With *Gigartina stellata*, both the upper and the lower limits were higher at Beer than those shown by Evans.

NITROGEN ESTIMATIONS ON *ULVA*

In an attempt to determine if the dominance of *Ulva lactuca* on the *Sabellaria* colonies was in any way reflected in a change in the nitrogen-content of the *Ulva*, some estimations of the total nitrogen-content of *Ulva* samples were carried out. The material was collected at Beer in June 1956 and the samples were treated immediately on return to London. *Ulva* was collected from areas of 100 cm^2 and, after a quick washing to remove sand, etc., was dried to constant weight in an oven at 100° C. The percentage nitrogen content of the samples, on a total dry-weight basis, was then determined by means of the micro-Kjeldahl method. Eight samples of *Ulva* growing on *Sabellaria* colonies, and two samples not on colonies, were estimated. The mean of the dry weights of *Ulva* per 100 cm^2 on the colonies was 1·37 g, and off the colonies was 1·01 g. The mean percentage nitrogen on a total dry-weight basis was 0·68% on the colonies, standard deviation 0·21, and off the colonies was 2·64%, standard deviation 0·14. These results show a slightly higher yield per unit area, but a lower nitrogen content per unit dry weight on the colonies. This may indicate a more rapid growth of *Ulva* on the colonies. In both cases the values are considerably less than those quoted by Milner (1953), who gives a value of 4·87% on a dry-weight-less-ash basis, i.e. 3·95% on a total dry-weight basis.

DISCUSSION

It seems clear from the species maps that, at Beer, *Ulva lactuca* dominates large areas only if they are colonized by the *Sabellaria alveolata* worms. On the *Sabellaria* colonies the *Ulva* is different in appearance from that off the colonies, in that the plants on the colonies are smaller in height and much more tufted at the base than the forms growing off the colonies. This dominance of *Ulva lactuca* on the colonies of *Sabellaria* may be related to one or more of three possibilities. (i) The *Sabellaria* colonies form a very unstable substratum and the larger algae such as the fucoids cannot become established on them. (ii) Due to the presence of some factor produced by the *Sabellaria* worms, the growth of *Ulva* on the colonies is very rapid and hence the *Ulva* soon forms a complete cover to the exclusion of other algae. (iii) The colonies produce something that is toxic to most algae but not to *Ulva*.

The first factor does not seem to be likely as *Gigartina* plants reach considerable size where they are the dominant plants on the *Sabellaria* colonies and these probably grow at a comparable rate to the fucoids. Further, in some areas the fucoids do encroach slightly on to the *Sabellaria* colonies and are thus able to grow on the colonies if they get a chance. By the same reasoning, the idea that there may be some toxin released must be excluded. It is, however, difficult to show any definite evidence for the second factor and the nitrogen estimations did not give any explanation here.

Cotton (1910) noted the good growth of *Ulva* in quiet brackish water that was strongly polluted, he also found that *Ulva* could assimilate high levels of nitrate and ammonia. Arber (1901) asserted that abnormally high levels of nitrates caused inhibition of carbon-assimilation in *Ulva* cultures. Letts & Richards (1911) found the addition of filtered sewage, up to 10%, gave a considerable increase in growth, growth being estimated by the increase in surface area. They also found that the increase was greater than that brought about by the addition of nitrate and phosphate. Foster (1914) found that *Ulva* grew quite well on ammonium nitrate, urea and acetamide. In all this work no attempts were made to prevent, or even reduce, bacterial contamination.

Kylin (1942) found that glucose, ascorbic acid and heteroauxin, at certain concentrations, all increased the rate of division in *Ulva* sporelings. In 1943 he commented on the stimulatory effects of thiamin and of biologically active substances in the surface layers of the sea, especially if the water was collected in regions of dense algal growth. Later (1945) he found that the effects of these biologically active substances and the effects of iron and manganese were additive. Again, however, these cultures were contaminated.

Cotton (1911) commented on the abundance of *Ulva* on mussel beds and pointed out that, under these conditions, sporing tends to be reduced and the main form of reproduction is vegetative. As previously stated, I have noted that the plants on the *Sabellaria* colonies at Beer are small in extent and very tufted at the base. This may indicate the extensive vegetative reproduction mentioned by Cotton.

Gigartina occurs both on the Greensand and on the *Sabellaria* colonies and thus appears not to be in any way affected by the *Sabellaria* worms, although it should be pointed out that the highest level at which *Gigartina* occurred at Beer was on a *Sabellaria* colony. As was previously stated, the two species of *Fucus* do not show any divergences from the normal zonation found on the south coast of England. Both species occur on all types of substratum.

Enteromorpha shows a wide distribution throughout the intertidal zone, but, as this genus is known to be greatly influenced by local conditions such as the presence of fresh water and high nitrogen sources, the wide distribution is not surprising. Although *Enteromorpha* was very abundant on the chalk boulders, it occurred also at the same levels on the Greensand, and, furthermore, its distribution was correlated with that of fresh water which ran from the base of the cliffs, and thus, in this case, there was no correlation of distribution with substratum.

From this investigation it is concluded that the distribution of the main marine algal species in Beer Bay was quite normal for an exposed area on the south coast of England and, with the exception that *Ulva lactuca* was only dominant over large areas where these were colonized by the reef-building worm *Sabellaria alveolata*, there was no effect of substratum on the distribution of the algal species.

I wish to thank Dr G. E. Fogg for his helpful criticism of this work, Mr J. H. Belcher for help with the nitrogen estimations, and Miss J. M. Kain for help in the construction of the level used on the transects.

SUMMARY

The intertidal zonation of the dominant algal species in Beer Bay, south-east Devon, was examined in relation to substratum by means of plane-table maps and transects. The dominance of *Ulva lactuca* on colonies of the reef-building worms *Sabellaria alveolata* was noted, but the distributions of *Fucus vesiculosus*, *F. serratus*, *Gigartina stellata* and *Enteromorpha* spp. showed no correlation with substratum.

REFERENCES

ANAND, P. L., 1937. An ecological study of the Algae of British Chalk cliffs. *J. Ecol.*, Vol. 25, pp. 153–88, 344–67.

ARBER, E. A. N., 1901. On the effect of nitrates on the carbon assimilation of marine Algae. *Ann. Bot., Lond.*, Vol. 15, pp. 669–81.

COTTON, A. D., 1910. On the growth of *Ulva latissima* L. in water polluted by sewage. *Kew Bull.*, 1910, pp. 15–19.

—— 1911. The ecology of *Ulva. 7th Rep. Royal Commission on Sewage Disposal.* Appendix 3, pp. 121–43.

DEWEY, H., 1948. *British Regional Geology South West England.* D.S.I.R. Geological Survey.

EVANS, R. G., 1947. The intertidal ecology of selected localities in the Plymouth neighbourhood. *J. mar. biol. Ass. U.K.*, Vol. 27, pp. 173–218.

FOSTER, G. L., 1914. Indications regarding the source of combined nitrogen of *Ulva lactuca. Ann. Mo. bot. Gdn*, Vol. 1, pp. 229–35.

GRUBB, V. M., 1936. Marine algal ecology and the exposure factor at Peveril Point, Dorset. *J. Ecol.*, Vol. 24, pp. 392–423.

JUKES-BROWNE, A. J., 1904. The cretaceous rocks of Britain, Vols. 1 and 2. *Mem. geol. Surv. U.K.*

KNIGHT, M. & PARKE, M., 1950. A biological study of *Fucus vesiculosus* L. and *F. serratus* L. *J. mar. biol. Ass. U.K.*, Vol. 29, pp. 439–514.

KYLIN, H., 1942. Über den Einfluss von Glucose, Ascorbinsäure und Heteroauxin auf de Keimlinge von *Ulva* und *Enteromorpha. K. fysiogr. Sällsk. Lund Förh.*, Bd. 12, pp. 1–14.

—— 1943. Über die Ernährung von *Ulva lactuca. K. fysiogr. Sällsk. Lund Förh.*, Bd. 13, pp. 1–13.

—— 1945. Weitere Angaben über die Ernährung von *Ulva lactuca. K. fysiogr. Sällsk. Lund Förh.*, Bd. 15, pp. 1–5.

LETTS, E. A. & RICHARDS, E. H., 1911. On green sea-weeds in relation to the pollution of the waters in which they occur. *7th Rep. Royal Commission on Sewage Disposal*, Appendix 3, pp. 72–120.

MEYER, C. J. A., 1874. The Cretaceous rocks of Beer Head. *Quart. J. geol. Soc. Lond.*, Vol. 30, pp. 369–93.

MILNER, H. W., 1953. The chemical composition of Algae. Algal culture from laboratory to pilot plant. *Publ. Carneg. Instn*, No. 600, pp. 285–302.

NEWTON, L., 1931. *A Handbook of the British Seaweeds.* London: British Museum (Natural History).

PARKE, M. W., 1953. A preliminary check-list of British marine Algae. *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 497–520.

ROWE, A. W. & SHERBORNE, C. D., 1903. The zones of the White Chalk of the English Coast. III. Devon. *Proc. Geol. Ass., Lond.*, Vol. 18, pp. 1–51.

WOODWARD, H. B. & USSHER, W. A. E., 1911. Geology of the country near Sidmouth and Lyme Regis. *Geological Memoir*, No. 326, and No. 340.

J. mar. biol. Ass. U.K. (1957) 36, 529–538
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SPECTRAL COMPOSITION OF THE LIGHT OF POLYNOID WORMS

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(Text-figs. 1–14)

The spectral composition of the light of only one marine animal is known, viz. the ostracod *Cypridina hilgendorfii* (see Coblentz & Hughes, 1926; Eymers & van Schouwenburg, 1937). It is desirable to have spectral energy curves for the light of other animals in order to relate these to the spectral sensitivity of photoreceptors, and to calculate total radiant energy in the visible range. To further these ends I have measured the spectral energy distribution of the light of some polynoid worms.

The luminescence of polynoids originates in the elytra or scales covering the dorsal surface. The photocytes form a single epithelial layer on the lower surface of the centre of the elytrum, and the light shines through the elytrum, which is clear and non-pigmented over the luminescent tissue. The light appears emerald-green in colour, and presumably has an emission peak somewhere between 510 and 530 mμ. In the normal response the light is emitted in brief discontinuous flashes, some 0·1 sec. in duration (Nicol, 1953, 1957*a*). Repeated or strong shocks sometimes produce a prolonged glow, in which the luminescence tends to become exhausted. Separate flashes vary greatly in intensity, owing to facilitation and fatigue (Nicol, 1954). The problem, then, is to determine the spectral composition of brief flashes of weak green light, varying greatly in intensity. The procedure adopted is described below.

MATERIAL AND METHODS

Four species of polynoid worms were used, viz. *Lagisca extenuata*, *Gattyana cirrosa*, *Polynoë scolopendrina* and *Harmothoë longisetis*. Elytra were removed from a worm under $MgCl_2$-narcosis, and washed in sea water. An isolated elytrum was mounted in a glass and Perspex moist chamber over a pair of electrodes, and the chamber was sealed with a glass coverslip. This arrangement allowed the light to be detected from above and below the scale. Below the chamber lay an 11-stage photomultiplier (E.M.I. no. 6260), with a 45 mm end-window; above, a 14-stage photomultiplier (E.M.I. no. 6685), with a 9 mm end-window. The two photomultipliers were connected to separate cathode-ray oscilloscopes, and photographic records of the luminescent responses were made on moving paper. The arrangement is illustrated in Fig. 1.

The lower photomultiplier was used to provide an index of flash intensity. A coloured filter was placed between the specimen and the upper photomultiplier, which was used to measure the light transmitted by a certain spectral band. The coloured filters were Ilford's spectrum set, nos. 601 to 608, covering the visible spectrum; curves are given in *Ilford colour filters*, published by Ilford Ltd. In some instances a neutral filter (Chance's neutral glass ON 31 or 32) was placed over the lower photomultiplier to prevent saturation from very bright flashes. This arrangement allowed very bright responses to be recorded by the lower photomultiplier, without altering voltage on the tube, in those instances when measurements were being made with coloured filters 606–608 in front of the upper photomultiplier. I determined the transmission of my set of filters with a spectrophotometer (Unicam SP 500).

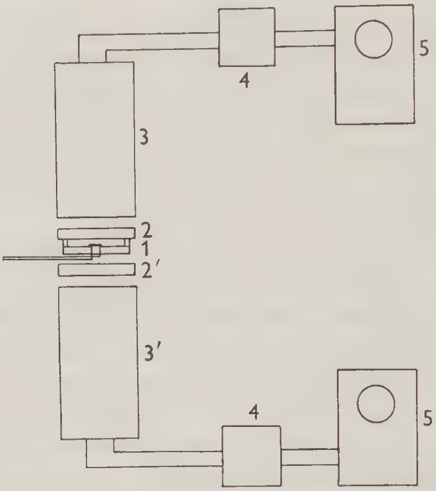


Fig. 1. Diagram of apparatus. 1, moist chamber containing elytrum and electrodes; 2, coloured spectral filter; 2′, neutral filter; 3, photomultiplier 6685; 3′, photomultiplier 6260; 4, d.c. amplifiers; 5, cathode-ray oscilloscopes.

Both photomultipliers had Cs–Sb photocathodes, with high sensitivity in the violet, and very low sensitivity in the red. The spectral sensitivity of photomultiplier no. 6685 was determined by the National Physical Laboratory. Combined data for photomultiplier no. 6685 and coloured filters are given in Table 1.

The mains voltage was maintained constant for all equipment by a voltage stabilizer. Both photomultipliers were run off the same power-pack, which held steady voltage, as determined by periodic checks.

Repeat determinations, made with a constant light source, showed that measurements could be made on the oscilloscope screen with an accuracy of ±2%.

A series of observations was made with the several coloured filters on each elytrum. If the elytrum continued to respond with sufficient intensity, it was possible to do a run with all filters on a single elytrum. From two to eight runs were made with all filters for the elytra of each species. Since the elytra flash repetitively to stimulation, each photographic record reveals a series of flashes (10 or more), a selected number of which was measured. The conclusions are based on about 150 photographic records and an analysis of some 1000 flashes.

Room temperatures during the measurements varied from 18° to 19° C.

TABLE 1. CERTAIN CHARACTERISTICS OF ILFORD SPECTRUM FILTERS AND E.M.I. PHOTOMULTIPLIER NO. 6685

		Maximal transmission			
Ilford filters	Filter range (mμ)	λ	%	Representative wavelength (mμ)	Area of curve $S_\lambda T_\lambda$*
Violet 601	400–485	440	16·8	433	230·0
Blue 602	440–495	465	7·1	465	48·0
Blue-green 603	470–525	490	13·5	490	81·0
Green 604	500–540	515	7·8	514	32·1
Yellow-green 605	525–570	545	6·7	548	24·43
Yellow 606	560–610	575	7	573	11·0
Orange 607	575–700	600	18·2	595	23·26
Red 608	610–700	700	81	661	5·6

* S, sensitivity of photomultiplier. T, transmission of filter.

OBSERVATIONS

ESTIMATION OF ACCURACY, USING A KNOWN LIGHT SOURCE

In order to evaluate the accuracy of the method, light from a known source was measured by means of the Ilford spectrum filters and photomultiplier no. 6685. The light was provided by a substandard lamp (colour temperature 2360° K), and was passed through a yellow-green filter (Chance OGR 2). Measurements of the response were made on the oscilloscope-face for each of the spectral filters nos. 601–608. To reduce intensities, a Chance neutral density glass (no. ON 31) was used in conjunction with Ilford filters nos. 603–607. Relative transmission values of the green filter OGR 2 were determined with a spectrophotometer (Unicam SP 500). The curves in Fig. 2 show the actual transmission of filter OGR 2 (curve A), and the calculated spectral composition ($J_\lambda T_\lambda$) of the light reaching the photomultiplier (curve B). The circles in Fig. 2 are the first estimations of relative spectral energy, made with spectral filters, photomultiplier and oscilloscope.

Most of the filters have rather broad transmission bands, and the sensitivity of the photomultiplier changes greatly over the visual spectrum. The values for the combination $S_\lambda T_\lambda$ are based on the assumption of an equal energy spectrum. Light having a pronounced spectral peak will differ from an equal energy spectrum in its effects at various wavelengths according to the characteristics of each factor in the combination $E_\lambda S_\lambda T_\lambda$. To predict the results, an approximate energy curve was drawn from the points in Fig. 2, and energy levels ($E_{\lambda A}$) from this curve were used to calculate a series of values for $\int E_{\lambda A} S_\lambda T_\lambda$. These values were then used to correct the results, in the manner outlined in the next section. The final corrected estimations of relative spectral composition, based on direct measurements, are represented by the circles in Fig. 3, which also gives the calculated spectral composition of artificial light ($\mathcal{J}_\lambda T_\lambda$). Values for $\mathcal{J}_\lambda$ were taken from Skogland (1929). There is reasonably good agreement between predicted and measured values. These measurements, of course, were made under optimal conditions (steady light intensity and absence of background noise).

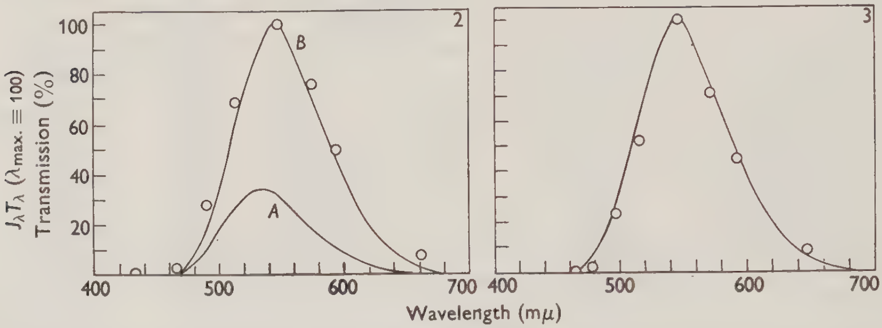


Fig. 2. Curve A: transmission (%) of a green filter. Curve B: calculated relative spectral energy of light from a tungsten lamp (2360° K), transmitted through a green filter (Chance OGR 2) ($\mathcal{J}_\lambda T_\lambda$). Circles are first measurements of relative spectral energy made by means of coloured filters and photomultiplier no. 6685.

Fig. 3. Curve as in Fig. 2B (calculated relative spectral energy of artificial light—source 2360° K plus Chance OGR 2). Circles are corrected measurements of relative spectral energy.

MEASUREMENTS OF THE LIGHT FROM POLYNOID ELYTRA

First approximate results obtained by measuring the light emission of four species of polynoids are given in Table 2, and the data are presented graphically in Figs. 4–7. Some representative photographic records are shown in Fig. 8. The data for an experiment on one animal (*Polynoë*) are given in greater detail in Table 3, to illustrate the calculations involved. Measurements for all four species of polynoids are pooled together to give the emission curve of Fig. 9. The measurements derived from the use of filters 602, 606, 607 and 608 are less reliable than the others, owing to high density of the filters (602, 606) and low sensitivity of the photomultiplier at long wavelengths (orange and red). The electrical response to red light which reached the photocathode was very small, and the results in the red region of the spectrum can be in error by a factor of 2. With the denser filters the transmitted light was equal to, or below, visual threshold. The main source of error lies in evaluating response height through the background noise at high amplification. This was

TABLE 2. FIRST APPROXIMATIONS FOR SPECTRAL ENERGY OF THE LIGHT OF POLYNOIDS

Averages of readings.

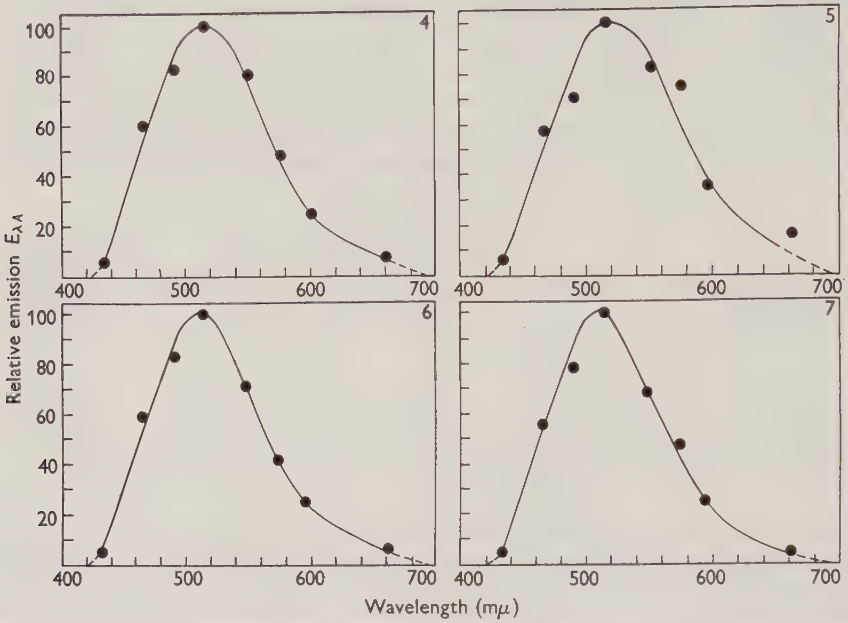
Filters	*Lagisca*	*Gattyana*	*Harmothoë*	*Polynoë*	Means, four species ($\lambda_{\text{max.}}$ as 100)
601	6·63	9·43	3·61	3·62	5·85
602	71·17	87·63	41·37	32·88	58·53
603	98·77	109·96	58·24	45·60	78·50
604	118·91	151·36	69·57	58·32	100
605	97·56	126·68	49·53	40·08	78·83
606	63·95	115·88	29·42	28·17	59·62
607	34·93	60·14	17·9	14·67	32·06
608	20·65	8·79	4·88	7·59	10·53

TABLE 3. CALCULATION OF THE RELATIVE ENERGY EMITTED BY AN ELYTRUM OF *POLYNOË* IN DIFFERENT SPECTRAL BANDS

Filters		Response (R_X)	Factor for photomultiplier sensitivity and filter transmittance $\left(\eta_X=\int S_\lambda T_\lambda\right)$	Relative energy $\times$ 10,000 (R_X/η_X)	Relative energy as percentage of maximum 515 mμ=100
Violet 601		0·1027			
		0·0833			
		0·0951			
	Mean	0·0937	230·0	4·07	6·28
Blue 602		0·1731			
		0·1912			
		0·1852			
		0·1905			
		0·1818			
		0·1931			
	Mean	0·1858	48·0	38·70	60·63
Blue-green 603		0·3808			
		0·3506			
		0·3818			
		0·3759			
		0·4100			
	Mean	0·3798	81·0	46·89	72·33
Green 604		0·2126			
		0·2136			
		0·2217			
		0·2068			
		0·1955			
		0·1982			
	Mean	0·2081	32·1	64·83	100
Yellow-green 605		0·1191			
		0·1040			
		0·1201			
		0·1002			
	Mean	0·1109	24·43	45·41	70·04
Yellow 606		0·0401			
		0·0344			
	Mean	0·0377	11·0	34·27	52·86
Orange 607		0·0367			
		0·0386			
	Mean	0·0376	23·26	16·17	24·94
Red 608		0·0356			
		0·0300			
	Mean	0·0328	5·6	5·86	9·04

particularly difficult in the weak responses obtained with the yellow, orange and red filters. Each point on the curves represents the mean of some thirty measurements.

Because of the broad transmission of the filters, the first approximate results for relative spectral emission were corrected as follows. The mean



Figs. 4–7. Curves showing relative spectral energy of light emitted by four species of polynoids, viz. *Lagisca* (Fig. 4), *Gattyana* (Fig. 5), *Harmothoë* (Fig. 6), and *Polynoë* (Fig. 7).

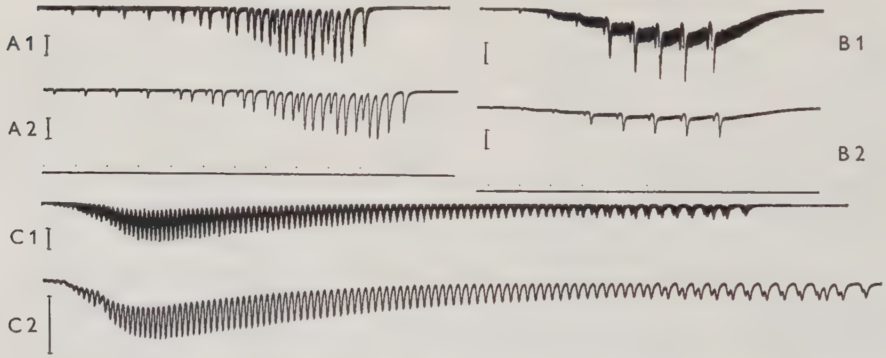


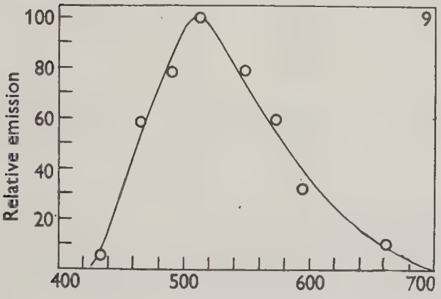
Fig. 8. Oscillograph records of the luminescent responses of *Harmothoë longisetis*, selected as examples. Records as follows: A 1, upper photomultiplier + blue filter 602; A 2, lower photomultiplier; B 1, upper photomultiplier + blue-green filter 603; B 2, lower photomultiplier; C 1, upper photomultiplier + yellow filter 606; C 2, lower photomultiplier. Relative amplifications shown by vertical lines. Electrical stimuli on lower lines of A 2 and B 2.

spectral energy curve of Fig. 9 was used to provide approximate values for radiant flux ($E_{\lambda A}$). With these values, curves were plotted for $E_{\lambda A} S_\lambda T_\lambda$ against λ (Fig. 11). Ratios were then obtained for each filter

$$\rho_1 = \frac{\int E_{\lambda A} S_\lambda T_{\lambda X}}{\int E_{\lambda A} S_\lambda T_{\lambda 604}},$$

which were used to predict the experimental results. Similar ratios were determined for measured responses

$$\rho_2 = \frac{R_X}{R_{604}}.$$



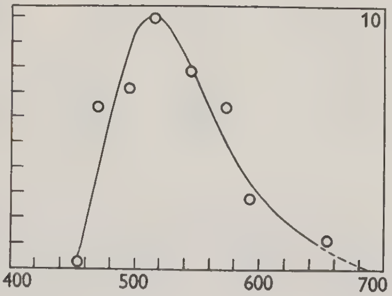


Fig. 9. Composite curve showing relative spectral energy of polynoid light (mean of determinations of four species, viz. *Lagisca*, *Polynoë*, *Harmothoë* and *Gattyana*). First approximate values.

Fig. 10. Composite curve showing relative spectral energy of polynoid light. Corrected results.

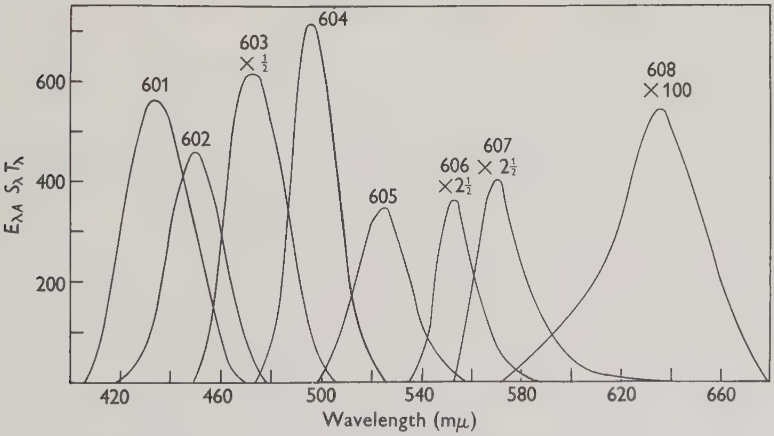


Fig. 11. Curves for combinations $E_{\lambda A} S_\lambda T_\lambda$ plotted against λ. Values for $E_{\lambda A}$ are taken from the curve in Fig. 9.

The ratios $\rho_3 = \rho_2/\rho_1$ were then used to correct the points on the spectral curve. New values for representative mean wavelengths were calculated from the centre of gravity of curves $E_{\lambda A} S_\lambda T_{\lambda X}$. The procedure is illustrated in more detail in another paper dealing with the light of *Chaetopterus* (Nicol, 1957*b*). The calculations are assembled in Table 4, and the corrected results are plotted against λ in Fig. 10.

The relative spectral energy curves (Figs. 4–7, 9, 10) are asymmetrical, with peaks at *ca.* 515 mμ (510–520 mμ). Most of the light emitted is in the blue-green, and there is very little in the violet and red.

TABLE 4. CALCULATION OF RELATIVE SPECTRAL COMPOSITION OF THE LIGHT OF POLYNOIDS

Filter	$E_{\lambda A} S_\lambda T_\lambda = \zeta_X$	$\zeta_X/\zeta_{604} = \rho_1$	R_X	$R_X/R_{604} = \rho_2$	$\rho_2/\rho_1 = \rho_3$	Corrected values $Q_X \rho_3$	$Q_X \rho_3$ $\lambda_{515 m\mu} \equiv 100$	Mean λ (from curves $E_{\lambda A} S_\lambda T_\lambda$) (m$\mu$)
601	35·617	1·145	0·13386	0·4189	0·366	2·13	2·14	455
602	24·616	0·792	0·279648	0·875	1·105	64·38	64·67	470
603	67·566	2·173	0·632934	1·981	0·912	71·26	71·59	494
604	31·10	1	0·319523	1	1	99·54	100	515
605	18·625	0·599	0·191678	0·599	1	78·46	78·82	545
606	5·873	0·189	0·065296	0·204	1·079	64·05	64·35	572
607	8·133	0·262	0·074223	0·232	0·885	28·24	28·37	592
608	0·504	0·016	0·005869	0·018	1·125	11·79	11·84	653

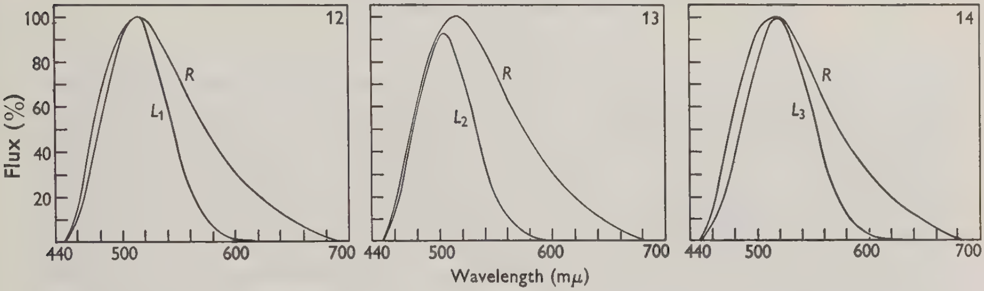
DISCUSSION

The light emitted by four different polynoids is emerald green in colour, with maximal energy content at *ca.* 515 mμ. The spectral energy curves for the several species of polynoids examined are closely similar within the limits of experimental error, and may well be identical. The identity of physiological mechanisms controlling luminescence in polynoids suggests that the same identity may exist in biochemical mechanisms. However, this argument cannot be pressed very far in view of the known differences existing in the colour of light emitted by other closely related animals, viz. different species of lampyrid beetles (Harvey, 1952).

In Figs. 9 and 10 all results have been combined to give a composite relative spectral energy curve for polynoid light. Few data are available from other marine animals for comparison. Maximal emission in *Cypridina* lies at 480 mμ. The light of *Chaetopterus* appears blue, with a similar spectral range to that of *Cypridina*; its maximum lies in the region 465 mμ. A green light emitted by the teleost *Malacocephalus* (apparently bacterial in origin) has a maximum at *ca.* 510 mμ (references cited by Harvey, 1952). Harvey (1955) lists the colours of light emitted by other species.

The luminous efficiency of radiation is given by the ratio of total luminous flux to total radiant flux (Harvey, 1940). In the present instance the difficulty lies in choosing a significant visibility curve, since there are no experiments or observations to show the biological significance of light emission among polynoids, i.e. what photoreceptors polynoid light is accustomed to excite. As a first attempt I have determined the luminous efficiency for human scotopic vision (from C.I.E. values for relative efficiency of radiation (scotopic vision)). The relevant curves are given in Fig. 12. Luminous efficiency for polynoid light is 61%. This means that the worms appear 39% less bright than they would be if all the energy were concentrated at that wavelength (505 mμ) for which the dark-adapted human eye is most sensitive.

Visibility curves for various invertebrates and lower vertebrates have maxima ranging from about 490 to 540 mμ (e.g. *Eledone*, 490 mμ; *Limulus*, 520 mμ; *Lepomis*, 540 mμ) (Graham & Hartline, 1935; Grundfest, 1932; Bliss, 1943). Vertebrate rhodopsins, occurring in marine coastal and surface fish, show absorption maxima around 500 mμ; porphyropsins, found in wrasse, trout, etc., have maxima around 520 mμ; deep-sea teleosts have visual pigments with absorption maxima around 480 mμ (Wald, 1952, 1953; Granit, 1955; Denton & Warren, 1956). The emission spectrum of polynoid light is fairly broad. Calculations based upon two generalized visibility curves, similar in shape to those for human scotopic vision, but having maxima at 490 and 520 mμ, give luminous efficiencies of 51 and 66% respectively (Figs. 13, 14). A narrow spectral emission curve, like that for *Cypridina* with its maximum at 480 mμ, will show much lower luminous efficiencies for visibility curves having maxima at longer wavelengths.



Figs. 12–14. Luminous efficiency of light emitted by polynoids. Curves for radiant flux (*R*) of polynoid light, and luminous flux based on human scotopic vision, $\lambda_{\text{max.}}$ at 505 mμ (L_1) (Fig. 12); luminous flux based on a theoretical visual curve with maximum at 490 mμ (L_2) (Fig. 13); and luminous flux based on a theoretical visual curve with maximum at 520 mμ (L_3) (Fig. 14).

I wish to thank Mr F. A. J. Armstrong for help in technique, and Dr E. J. Denton for advice concerning measurements and calculations of light intensity. Part of the apparatus used in this investigation was purchased with a grant from the Royal Society.

SUMMARY

The spectral composition of the light of four species of polynoid worms has been measured, viz. *Harmothoë longisetis*, *Gattyana cirrosa*, *Polynoë scolopendrina* and *Lagisca extenuata*. The method involved the use of coloured spectral filters and two multiplier phototubes. The spectral emission curves of the four species are similar. They are asymmetrical in shape, prolonged towards longer wavelengths, with maxima at about 515 mμ (510–520 mμ). Values for luminous efficiency are calculated (ratio of luminous flux to radiant flux); for human scotopic vision ($\lambda_{\text{max.}}$ at 505 mμ), efficiency is 61 %.

REFERENCES

Bliss, A. F., 1943. Derived photosensitive pigments from invertebrate eyes. *J. gen. Physiol.*, Vol. 26, pp. 361–7.

Coblentz, W. W. & Hughes, C. W., 1926. Spectral energy distribution of the light emitted by plants and animals. *Sci. Papers U.S. Bur. Standards*, Vol. 21, pp. 521–34 (no. 538).

Denton, E. J. & Warren, F. J., 1956. Visual pigments of deep-sea fish. *Nature, Lond.*, Vol. 178, p. 1059.

Eymers, J. G. & van Schouwenburg, K. L., 1937. On the luminescence of bacteria. III. Further quantitative data regarding spectra connected with bioluminescence. *Enzymologia*, Vol. 3, pp. 235–41.

Graham, C. H. & Hartline, H. K., 1935. The response of single visual sense cells to lights of different wavelengths. *J. gen. Physiol.*, Vol. 18, pp. 917–31.

Granit, R., 1955. *Receptors and Sensory Perception.* New Haven: Yale University Press.

Grundfest, H., 1932. The sensibility of the sun-fish, *Lepomis*, to monochromatic radiation of low intensities. *J. gen. Physiol.*, Vol. 15, pp. 307–28.

Harvey, E. N., 1940. *Living Light.* Princeton University Press.

—— 1952. *Bioluminescence.* New York: Academic Press.

—— 1955. Survey of luminous organisms: problems and prospects. In *The Luminescence of Biological Systems*, ed. F. H. Johnson, pp. 1–24. Wash., D.C.: Amer. Ass. Adv. Sci.

Nicol, J. A. C., 1953. Luminescence in polynoid worms. *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 65–84.

—— 1954. The nervous control of luminescent responses in polynoid worms. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 225–55.

—— 1957*a*. The luminescence of polynoids. Different modes of response in the elytra. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 261–9.

—— 1957*b*. Spectral composition of the light of *Chaetopterus*. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 629–42.

Skogland, J. F., 1929. Tables of spectral energy distribution and luminosity for use in computing light transmissions and relative brightnesses from spectrophotometric data. *U.S. Bur. Standards, Misc. Publ.*, no. 86, 23 pp.

Wald, G., 1952. Biochemical evolution. In *Modern Trends in Physiology and Biochemistry*, ed. E. S. G. Barron, pp. 337–76. New York: Academic Press.

—— 1953. The biochemistry of vision. *Ann. Rev. Biochem.*, Vol. 22, pp. 497–526.

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THE FEEDING AND CONDITION OF PLAICE LARVAE IN GOOD AND BAD PLANKTON PATCHES

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(Plate I and Text-figs. 1–3)

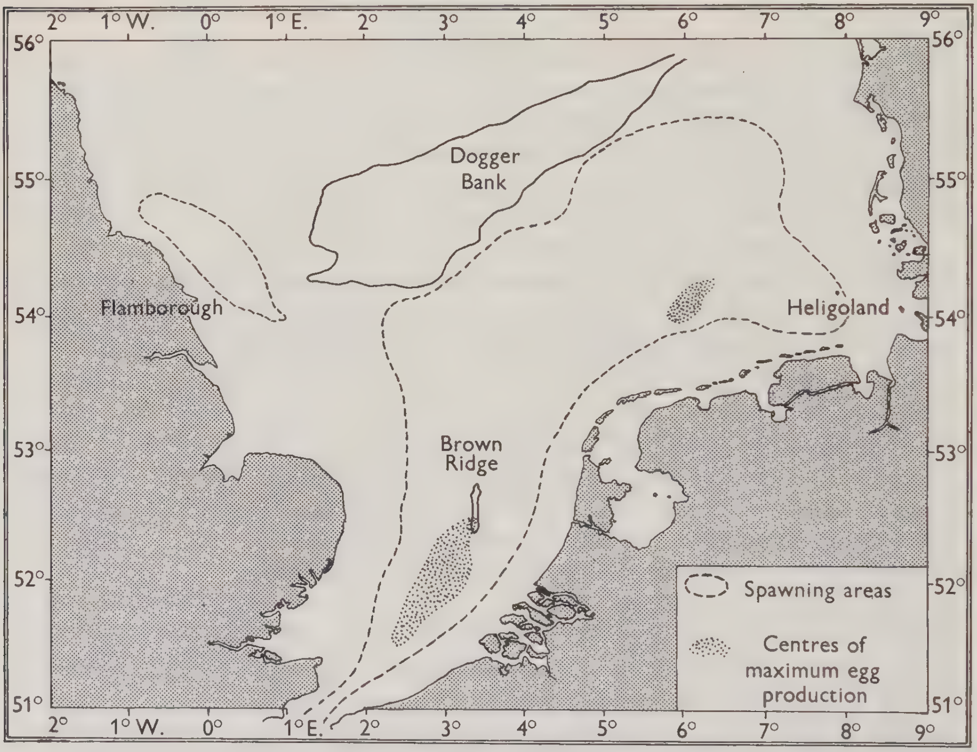
In spite of the high fecundity of many food-fish species, only a minute proportion of the total eggs spawned enter the fishery later on, as marketable adults. This principle is not in dispute. There is, however, a considerable division of opinion among fisheries biologists on the general shape of the mortality curve. Hjort (1914, 1926) suggested that the final size of a year-class of cod and herring is determined shortly after hatching, and is related to the larval food supply and drift. Sund (1924) supported this view. Johansen (1927) investigated yearly fluctuations in the abundance of fish larvae in Danish waters, and came to the conclusion '...that in cold winters, when there is a relatively marked outflow of comparatively fresh water from the Baltic, through the Belts and Southern Kattegat, the tiny plaice larvae fail to obtain sufficient nourishment and die of starvation *en masse*'. Rollefsen (1930) demonstrated the lethal effect of agitation on the vulnerable stages of the cod egg, and thought that wave action during incubation might strongly influence the early slope of the mortality curve. The observations of Soleim (1940, 1942) on herring larvae off the coast of Norway, and in the laboratory, led him to infer a critical starvation period following yolk resorption.

The reality, or otherwise, of a critical period in the early life history of marine fishes has recently been discussed by Marr (1956) using two sets of data; those of Sette (1943) on the Atlantic mackerel (*Scomber scombrus*) and of Sette & Ahlstrom (1948) and Ahlstrom (1954) on the Pacific sardine (*Sardinops caerulea*) of North America. Marr interpreted the Atlantic mackerel data as having a relatively constant survival rate with a brief high mortality at the age of 30–40 days, but no critical period at the time of yolk resorption. Although the Pacific sardine records show an apparent critical phase, Marr contested the reality of this result on the basis of inadequate sampling of the very young larval population.

As a complement to the studies of Simpson (1951) on the fecundity of North Sea plaice (*Pleuronectes platessa*) and the distribution of its eggs and larvae (in press), preliminary attempts were made during the 1955 spawning season, to follow the fate of plaice egg broods during their early life history. Bad weather limited activities, but some significant observations were made on the condition of plaice larvae in good and bad feeding conditions, relevant to the unsolved problem of early mortalities.

SAMPLING TECHNIQUE

The spawning season of the North Sea plaice lasts from late December until late March, with the peak of egg production occurring round about the end of January. The spawning area extends from the Straits of Dover to the south-east edge of the Dogger Bank and round into the Heligoland Bight (Text-fig. 1). There are two centres of maximum production: in the south, over the Brown Ridge, and in the north-east, off Heligoland. The Southern Bight spawning ground covers an area of some 8000 square miles; the Heligoland ground, some 2500 square miles. In addition, there is a minor spawning ground off Flamborough Head.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53°
52°
51°
Dogger Bank
Flamborough
Heligoland
Brown Ridge
Spawning areas
Centres of maximum egg production

Text-fig. 1. Plaice spawning grounds in the southern North Sea.

Marr (1956) has already stressed the importance of adequate coverage, in time and space, of spawning areas during mortality surveys, and criticizes the inference of Soleim (1942) regarding a critical phase, on these grounds. Plaice larvae from the Southern Bight drift in towards the Dutch and German coasts, parts of which are still mined. As winter weather interferes with plans for frequent surveys over such a large area of sea from a single research vessel, it was decided to attempt an intensive mortality study of the egg and larval population around a point marked by a floating radio buoy, rather than to assess the death-rate for the whole spawning area. The limitations of such a scheme were fully realized; in particular, the fact that the resultant mortality curve would not necessarily be typical for the whole area, even during the same period in time. Furthermore, it was not known whether a floating buoy, equipped with a 'tail' of sea drogues would continue to mark a pelagic plaice population in all weathers.

In late January 1955, a Heligoland larva net of 1·5 m diameter, with 60 meshes per inch silk, was used from R.V. *Sir Lancelot* south-west of the Brown Ridge, in search of an extensive patch of evenly scattered young plaice eggs. Such a patch was found, and a buoy, fitted with a short-range radio transmitter and a set of sea drogues, planted in the middle of it. By intensively sampling a close grid around the buoy in the middle of a big patch, it was hoped to overcome the problem set by differential water movement during gales, on the assumption that eggs and larvae moving out of grid range would be equally replaced.

Fair weather continued for a period of 12 days, during which time six grids of thirteen equally spaced stations per grid, were completed. Each grid covered an area of 1 square mile with the buoy as its centre. Two plankton hauls were made at each station, sampling after dusk to limit larval avoidance (Silliman, 1943; Bridger, 1956). Representative salinity samples and temperature records remained consistent throughout the 12-day period. Thereafter, strong south-westerly winds and a heavy swell shifted the buoy some 40 miles to the north-east in a week, and it was clear from the rapidly changing plaice egg population, supported by temperature and salinity records, that the buoy had moved off the original patch.

A second attempt was made in early March, and once again we were fortunate enough to have a 12-day period of tolerable weather. Another extensive patch of evenly distributed eggs and larvae was found south-west of the Brown Ridge, the buoy planted and a total of six grids completed before gales interfered with operations. In this case the grid had an extra station and covered an area of 5 square miles to give the ship plenty of room to manoeuvre into sampling positions during rough weather.

I do not intend to dwell at length on the difficulties attending this method of deriving a mortality curve. It is sufficient to say that in a shallow sea area, the chances of maintaining contact with the same fish brood throughout incubation and early larval development are not good. Differential water movements due to currents, tide and strong winds must have a profound effect on brood dispersion, rendering the study of diminishing numbers within a small body of water, an uncertain method of assessing mortality. A reasonable period of moderate weather is necessary for success.

Although these surveys failed to produce a mortality curve, they did focus attention on an anomalous situation very relevant to the death-rate of larval fish in the sea. There was a noticeable scarcity of plankton during the first series of grids in late January, coupled with generally poor larval condition and no indication of growth beyond the yolked stage. Plankton was abundant during the March grids; a good proportion of the larvae was robust and apparently advancing beyond the stage of final yolk loss. These differences made themselves felt during the cursory examination of plankton samples at sea. A more detailed study of formalin-preserved material was made in the laboratory.

DEVELOPMENT STAGES OF THE PLAICE

A plaice egg is provided with food, and its development rate during yolk utilization is, in normal circumstances, controlled by temperature (Apstein, 1909). Larval fish, on the other hand, may start taking food before the yolk is completely resorbed (Table 2). Experience in the laboratory suggests that the larval feeding rate affects the development rate at a given temperature. Thus, consistently successful feeders will reach metamorphosis long before their less voracious brethren in the same tank. Any attempt to assess the development rate of sea larvae without fore-knowledge of their feeding rates, must be very approximate. If the development rate varies from place to place in sea areas with patchy plankton, then it follows that over-all estimates of the early mortality rates must be similarly approximate.

Aurich (1941) produced a series of developmental stages for the plaice larva, based on the investigations of Dannevig (1897), Reibisch (1902) and Apstein (1909), which take into account the principal morphological changes between hatching and metamorphosis. Mr A. C. Simpson of this laboratory has amended Aurich's staging to give the following categories (Pl. I, figs. 1–5):

Stage 1. Yolk still present.
2. Yolk resorbed but notochord still straight.
3. Eyes still symmetrically placed. Notochord bent.
4. Eye started to move, but not yet reached edge of head.
5. Eye on or over the edge of the head.

I have further subdivided stages 1 and 2 as follows:

Stage 1. Yolk-sac stage.
(*a–c*) Divided into three categories according to the amount of yolk in the sac (indicated +) and also to the degree of curling of the gut and extent of ventral mesectodermal fusion (Shelbourne, 1956).

(*d*) A yolk spherule stage when the yolk has been reduced to a small sphere, usually located in the arch of the intestine.

2. (*a*) Early. The yolk completely resorbed but the distinctive cushion of tissue below the caudal axis not yet fully developed.

(*b*) Late. Caudal cushion distinct.

If food is scarce, the yolk spherule stage is succeeded by early stage 2 (*a*) when all the yolk has disappeared. In good food conditions, however, larvae may start heavy feeding whilst yolk is still present. The drain on the yolk is then interrupted and a yolk spherule may be carried over to stage 2 (*b*) when the caudal cushion is complete. For the purposes of this investigation, I prefer to regard stages 1 (*d*) and 2 (*a*) as a transitional group, lying between the 'yolked' and 'non-yolked' larvae of previous development studies. If a truly 'critical period' does occur in the life history of a brood of fish, it is surely at this point, when yolk reserves are exhausted and the larvae have to rely on external food sources.

COMPARATIVE ABUNDANCE OF PLANKTON

After removal of the plaice fraction, the net hauls at five representative stations per grid were analysed for zoo- and phytoplankton, using the Stempel pipette technique. The results are presented in Table 1. The estimates are to the nearest thousand after adjustment to an arbitrary total flowmeter reading of 200 revolutions per grid (40 rev./station). The species dealt with were

TABLE 1. THE COMPARATIVE ABUNDANCE OF PLANKTON IN THE JANUARY AND MARCH SAMPLES

		Zooplankton (thousands)				Phytoplankton (thousands)				
Date	Grid no.	Total copepods	Copepod nauplii	*Oikopleura* sp.	*Fritillaria* sp.	*Biddulphia* sp.	*Paralia*	*Bacillaria*	*Bellarochia*	*Navicula*
20. i. 55	1	30	—	—	—	—	—	—	5	—
21. i. 55	2	43	—	—	—	+	1	—	4	+
22. i. 55	3	36	—	—	—	—	11	—	—	—
24. i. 55	4	63	—	—	—	—	1	1	—	—
26. i. 55	5	36	—	—	—	1	4	—	—	+
1. ii. 55	6	21	—	+	+	1	+	—	—	—
8. iii. 55	7	66	2	6	1	49	4	7	—	5
10. iii. 55	8	94	2	7	1	358	5	73	7	23
16. iii. 55	9	193	4	10	4	1539	48	182	24	297
18. iii. 55	10	112	+	6	29	774	—	31	—	603
20. iii. 55	11	121	+	3	+	1070	—	57	—	255
22. iii. 55	12	174	2	5	+	1678	2	15	—	174

The numbers (to nearest thousand) are the total plankton counts for five representative stations on each grid, adjusted to a total flowmeter reading of 200 revs. + = present in small numbers.

common to both patches, but other diatoms were present in large numbers during the March cruises. The Heligoland larva net is not a quantitative sampling instrument for the smaller animals, e.g. copepod nauplii and very young appendicularians, nor for diatoms, but these organisms are caught in rough proportions to their abundance.

It is clear from Table 1 that over-wintering conditions prevailed in the first plankton patch. Adult copepods, too large to be used as food by plaice larvae, were fairly abundant, but copepod nauplii were not in evidence. A few *Oikopleura* made their appearance on the last grid in early February. Phytoplankton was generally scarce. By early March, the spring outburst was well under way; the plankton samples were characterized by the abundance and variety of both animal and plant species.

DIET OF PLAICE LARVAE

The quantity and nature of larval gut contents were recorded as part of the staging analysis. The results for both sets of grids are displayed in Table 2. In the absence of vulnerable animal food in January, the larvae ate plants only. The stomachs were never full nor the partly digested 'green food remains' (Lebour, 1919) identifiable, although fragments of diatom tests could be recognized. The value of plant food for larval growth is a debatable point. If sufficiently abundant, the large thin-walled diatoms, e.g. *Biddulphia* and *Coscinodiscus*, may help sustain larvae in the transition stage; when scarce, as in January, the energy expended during foraging activity may not be balanced by the calorific value of the plants eaten.

The spring diatom outburst is closely followed by a marked increase in the zooplankton, particularly the appendicularian fraction. The important part *Oikopleura* plays in the diet of plaice larvae from the southern North Sea, has already been discussed by Shelbourne (1953). When the pelagic larval phase and the appendicularian outburst coincide, these small, soft-bodied, vulnerable creatures are eaten to the exclusion of other zooplankton species. In the March plankton patch, they were present in numbers sufficient to support a considerable population of larval plaice, and, as will be seen later, to have a measurable effect on physical condition in the transition stage.

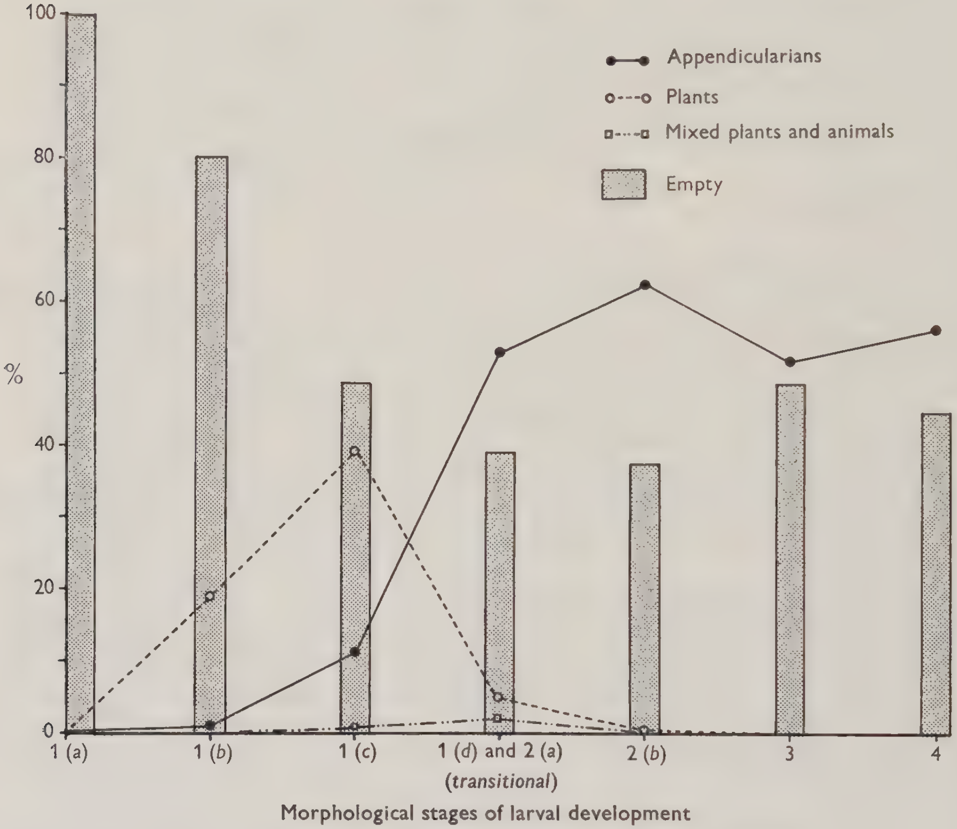
The feeding analysis for March is expressed graphically in Text-fig. 2. Larval feeding can start as early as the mid-yolk period, with diatoms the main food item until the end of the yolked stage, when appendicularians become important. The average larval mouth is probably too small to accept juvenile appendicularians before the transition stage is reached, even though the prey may be attacked during the yolk phase of the predator. Exclusive feeding on *Oikopleura* and *Fritillaria* continued throughout the subsequent pelagic life of the larva. The small proportion of stomachs containing mixed diatoms and appendicularians suggests that the change-over from plant to animal food is comparatively abrupt.

TABLE 2. THE GUT CONTENTS OF PLAICE LARVAE FROM BOTH PLANKTON PATCHES

Stage ...	Yolk ++ 1 (*b*)					Yolk + 1 (*c*)					Yolk spherule 1 (*d*)					Early 2 (*a*)				
	Total	*Oikopleura*	Plants	*Oikopleura* and plants	Empty	Total	*Oikopleura*	Plants	*Oikopleura* and plants	Empty	Total	*Oikopleura*	Plants	*Oikopleura* and plants	Empty	Total	*Oikopleura*	Plants	*Oikopleura* and plants	Empty
Grid 1	53	—	4	—	49	58	—	18	—	40	50	—	38	—	12	15	—	7	—	8
2	42	—	1	—	41	37	—	5	—	32	48	—	19	—	29	7	—	2	—	5
3	72	—	—	—	72	49	—	10	—	39	52	—	32	—	20	20	—	8	—	12
4	43	—	—	—	43	62	—	14	—	48	61	—	27	—	34	14	—	1	—	13
5	81	—	6	—	75	71	—	10	—	61	89	—	49	—	40	10	—	4	—	6
6	69	—	4	—	65	72	—	21	—	51	61	2	41	2	16	13	3	3	—	7
Totals	360	—	15	—	345	349	—	78	—	271	361	2	206	2	151	79	3	25	—	52
%		—	4·2	—	95·8		—	22·4	—	77·6		0·6	57·2	0·6	41·6		3·8	31·6	—	64·6
Grid 7	215	2	42	—	171	380	48	129	3	200	694	531	30	2	131	100	91	—	—	9
8	68	—	16	—	52	169	24	75	2	68	241	177	21	6	37	33	30	—	—	3
9	35	1	3	—	31	90	11	32	—	47	446	212	19	1	214	36	21	1	—	14
10	28	—	5	—	23	60	6	28	—	26	380	195	12	1	172	27	17	—	—	10
11	23	1	4	—	18	67	3	25	—	39	525	183	17	1	324	58	29	1	—	28
12	37	—	7	—	30	70	2	41	1	26	531	127	109	45	250	65	29	3	4	29
Totals	406	4	77	—	325	836	94	330	6	406	2817	1425	208	56	1128	319	217	5	4	93
%		1·0	19·0	—	80·0		11·2	39·5	0·7	48·6		50·6	7·4	2·0	40·0		68·0	1·6	1·2	29·2

Stage ...	Late 2 (*b*)					3					4				
	Total	*Oikopleura*	Plants	*Oikopleura* and plants	Empty	Total	*Oikopleura*	Plants	*Oikopleura* and plants	Empty	Total	*Okiopleura*	Plants	*Oikopleura* and plants	Empty
Grid 1–6			Nil					Nil					Nil		
Grid 7	146	114	—	—	32	32	18	—	—	14	8	2	—	—	6
8	89	75	—	—	14	44	37	—	—	7	3	3	—	—	—
9	142	44	—	—	98	46	8	—	—	38	4	3	—	—	1
10	63	39	1	—	23	23	7	—	—	16	3	2	—	—	1
11	157	77	1	—	79	59	22	—	—	37	8	1	—	—	7
12	129	102	1	1	25	32	30	—	—	2	8	8	—	—	—
Totals	726	451	3	1	271	236	122	—	—	114	34	19	—	—	15
%		62·1	0·5	0·1	37·3		51·7	—	—	48·3		55·9	—	—	44·1

The superimposed histograms represent the proportion of empty stomachs found at each development stage. With the onset of feeding activity, the 'empty' proportion drops, to reach a steady level, about 40%, for the post-yolk stages. The samples used in this analysis were caught between 18.00 h and midnight. In an earlier paper on larval feeding habits (Shelbourne, 1953), I followed the normal decline in feeding activity during the same period of darkness, and from these earlier records have calculated the mean proportion of empty stomachs as 34%. The fairly consistent level of emptiness during the post-yolk stages in March 1955 can therefore be explained by diurnal changes in the feeding rate, under the influence of light, rather than by scarcity of suitable food.



Text-fig. 2. Gut contents of plaice larvae from a good plankton patch.

THE CONDITION OF TRANSITION STAGE LARVAE

There was an association between food supply and the condition of the transition-stage plaice larvae. The problem of measuring larval condition was tackled by clearing and photographing five selected larvae (Pl. I, figs. 6–10), ranging from very thin to very robust, enlarging the prints to microscope image size, and using these prints as visual standards in the subsequent catch analysis.

The guiding principle in assessing robustness was the relationship of the soft parts (gut and muscular axis) to the cartilaginous head structure. A thin larva thus appeared ill-proportioned, with a prominent head and a reduced trunk. A robust larva, on the other hand, had balanced proportions, with a heavily muscled trunk, conspicuous gut and a far less prominent head.

Although the standards were somewhat subjective, the difference between larval condition during the January and March grids, is obvious enough (Table 3). The figures for each grid are the adjusted totals from two hauls taken at each station on the grid. The adjustment factor (for volume of water filtered) was reached by dividing the mean flowmeter revolutions per grid by the actual flowmeter reading. In this way one can compare the larval numbers from each grid on a particular patch, and the adjusted total of larvae examined per patch does not differ markedly from the actual total. The mean flowmeter reading for patch 1 differed from that of patch 2. The patch totals are not therefore adjusted to one another, but the condition compositions, dealing with proportions, are comparable. These proportions are expressed graphically in Text-fig. 3.

Plaice larvae were not very abundant in January. Half those at the beginning of the transition stage (end of yolk stage) were in medium condition, the other half thin. There were no robust or very robust individuals. Larvae caught at the end of the transition stage showed a distinct trend towards thinness; yolk reserves had been exhausted and soft structures reduced during continued foraging activity.

In contrast, about half the early transitional larvae from the good plankton patch were robust or very robust, indicating profitable feeding before complete yolk loss. In the same good food supply, 90% of the larvae leaving the transition stage were robust or very robust; fatter than their slightly younger contemporaries, not thinner as in January. There were no late stage 2 larvae in the poor plankton patch. In reasonable conditions for survival, one would expect this group to have appeared in the plankton by early February, accompanied by definite signs of increasing robustness in the late transitional stage, a necessary prelude to the formation of the characteristic caudal cushion. In the absence of these signs I am tempted to conclude that starvation conditions prevailed in the January patch and that survival was low. Roughly one-third of the late stage 2 larvae caught in the second plankton

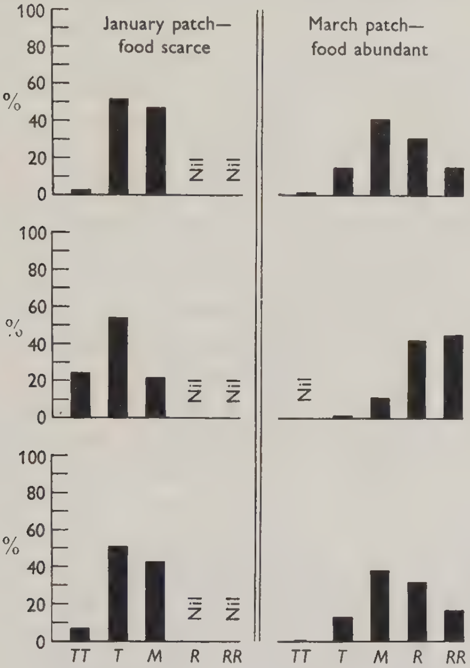
TABLE 3. ADJUSTED NUMBERS PER DEVELOPMENT STAGE OF PLAICE LARVAE, WITH A CONDITION ANALYSIS OF THE TRANSITIONAL STAGES

						Condition of larvae with yolk spherule						Condition of early stage 2 (*a*) larvae								
Date 1955	Grid no.	Yolk + + + 1 (*a*)	Yolk + + 1 (*b*)	Yolk + 1 (*c*)	Yolk spherule 1 (*d*)	Very thin	Thin	Medium	Robust	Very robust	Early stage 2 (*a*)	Very thin	Thin	Medium	Robust	Very robust	Late stage 2 (*b*)	Stage 3	Stage 4	Stage 5
Plankton patch 1. Mean temp. 6·8° C. Mean salinity 35·2 %																				
20. i.	1	45	45	49	43	3	16	24	—	—	13	6	7	—	—	—	—	—	—	—
21. i.	2	27	41	36	44	1	31	12	—	—	7	3	4	—	—	—	—	—	—	—
22. i.	3	63	61	42	45	2	17	26	—	—	16	4	9	3	—	—	—	—	—	—
24. i.	4	36	56	81	80	—	51	29	—	—	18	1	10	7	—	—	—	—	—	—
26. i.	5	94	96	84	104	2	48	54	—	—	11	2	8	1	—	—	—	—	—	—
1. ii.	6	62	66	68	58	—	29	29	—	—	13	3	4	6	—	—	—	—	—	—
Totals		327	365	360	374	8	192	174	—	—	78	19	42	17	—	—	—	—	—	—
Condition composition of transitional larvae (%)						2·1	51·4	46·5	—	—		24·4	53·8	21·8	—	—				
Plankton Patch 2. Mean temp. 4·1° C. Mean salinity 35·0 %																				
8. iii.	7	200	226	399	729	2	143	303	202	79	105	—	2	13	42	48	153 (45)	34	8	—
10. iii.	8	56	73	181	258	2	56	119	48	33	35	—	—	1	15	19	95 (33)	47	3	—
16. iii.	9	36	39	99	490	1	89	211	134	55	40	—	—	10	20	10	156 (54)	51	4	—
18. iii.	10	30	27	57	361	—	25	143	131	62	26	—	1	2	13	10	60 (30)	22	3	—
20. iii.	11	25	19	56	436	1	46	155	159	75	48	—	2	4	17	25	130 (47)	49	7	—
22. iii.	12	45	39	74	557	—	47	236	198	76	68	—	—	6	29	33	135 (49)	34	8	—
Totals		392	423	866	2831	6	406	1167	872	380	322	—	5	36	136	145	729 (258)	237	33	—
Condition composition of transitional larvae (%)						0·2	14·4	41·2	30·8	13·4		—	1·6	11·2	42·2	45·0				

The bracketed numbers in the late stage 2 (*b*) column represent larvae with a vestige of yolk still remaining in the body cavity.

patch had a vestige of yolk in the body cavity, a sure sign of better conditions for larval growth and survival.

There can be no doubt that a strong correlation must exist between larval condition and food supply, when other environmental factors lie within the limits of tolerance. During sampling the only hydrographic observations made were of temperature and salinity. The mean sea temperature was probably more favourable in January (6·8° C), than in March (4·1° C), and there is no evidence from previous work that the small salinity difference between patches (35·2‰: 35·0‰), could have such a profound effect on larval condition. However, there are growing indications of variable condition among developing plaice eggs caught at sea, and it is therefore unwise to assume that the January and March larvae of this investigation started life as embryos with uniform viabilities.



Text-fig. 3. Condition of transitional plaice larvae from good and bad plankton patches: *TT*, very thin; *T*, thin; *M*, medium; *R*, robust; *RR*, very robust. *Upper*, end of yolk stage, 1 (*d*). *Middle*, beginning of post-yolk stage, 2 (*a*). *Lower*, total transition stages, 1 (*d*) + 2 (*a*).

DISCUSSION

Many dangers beset the newly emerged marine fish larvae, not the least of which is osmosis. The mechanism of water and salt regulation is well known for the adult teleost, where its efficiency depends on the development of gills and chloride secreting cells, a completed circulatory system and a virtually water and salt proof skin (Krogh, 1939). Only the gill and oral membranes are permeable. Cod and plaice larvae (representing the pelagic marine group) lack these essential structures up to the stage of complete yolk loss (Shelbourne, 1956). The integument is extremely thin over the whole of its surface, and a considerable amount of energy must be expended, in the form of osmotic work, to maintain the internal environment against the osmotic gradient. The larval salt regulatory mechanism is not yet fully understood, but it seems likely, from these integumental differences, that the osmotic hazard facing the embryo and larva will be much greater than that facing the adult. Several factors, both external and internal, can upset the efficient functioning of the larval integument as a barrier to the passage of salts and water. In tanks, a rapid, though slight drop in the pH of the water is followed by coma, dehydration and death; a sudden water renewal, intended to benefit, can have the opposite effect. Starving larvae, lacking sufficient food reserves to meet osmotic needs, have little resistance, and once an 'osmotic breach' has been made, the larva is doomed. Opaque patches, often mistaken for fungal or bacterial growth, appear in the head and tail regions; the larva sinks, vibrates feebly for a short time and dies.

The problematical 'critical period' in the early life history of marine fishes can be explained in these simple physiological terms, and in natural conditions, a critical food supply in the transitional stage of development, is likely to produce local catastrophe. Such disasters would not necessarily be evident from spot samples; dying larvae sink rapidly after loss of osmotic control, and are probably snapped up by a multitude of hungry benthic scavengers. An adult marine fish, with its considerable food reserves, can survive lengthy lean periods. Its early larva is much more dependent on a day-to-day supply from external sources. Should they fail, then death is not far distant.

Throughout a spawning period, in seas characterized by very uneven plankton distribution in time and space, it is reasonable to suppose that the fates of larval populations vary considerably, from local catastrophe in food conditions below subsistence level, to local prosperity where plankton is rich. These variations would inevitably be masked in the mean mortality curve for a large area. Given moderate weather, plankton patches would seem to be better suited to investigations on the 'critical period' in the life history of fish larvae, than vast spawning areas.

SUMMARY

In January and March, during the 1955 plaice spawning season, plankton samples were collected with a Heligoland larva net at stations on a grid around a floating radio buoy in the southern North Sea. There was a decided scarcity of suitable food for plaice larvae in the January patch, and this famine was

reflected in the deteriorating physical condition of those larvae caught at the transition stage of development, when yolk reserves were becoming exhausted and an adequate external food supply essential. By March, the spring plankton outburst was in full swing. The condition of transitional larvae improved in this good food patch. Feeding started about the mid-yolk phase, mainly on plants. By the time most of the yolk had been resorbed, the appendicularians *Oikopleura* and *Fritillaria* had become the principal food items, and remained so throughout pelagic larval life.

Accurate mortality estimates during transient phases of development, depend largely on an understanding of the way in which the feeding rate affects the progress of larval development at a given temperature. Without this knowledge, the present data cannot be used to calculate mortality rates in the two plankton patches, but there are indications that in January, food was too scarce to support the growth and continued survival of the larval population, whereas in March, sufficient food was available for growth in the transitional stage, thus increasing the prospects of post-yolk survival.

There is reason to suppose that in local starvation conditions, larval control over their own internal salt balance would be greatly reduced, by lack of energy reserves for osmotic work at the thin integument. Failing a rapid improvement in the food situation, death must inevitably follow, and such local catastrophes would be hidden in the mortality curves derived from surveys over vast spawning areas.

REFERENCES

AHLSTROM, E. H., 1954. Distribution and abundance of egg and larval populations of the Pacific sardine. *Fish. Bull., U.S.*, Vol. 56, No. 93, pp. 83–140.

APSTEIN, C., 1909. Die Bestimmung des Alters pelagisch lebender Fischeier. *Mitt. dtsch. SeefischVer.*, Bd. 25, pp. 364–73.

AURICH, H. J., 1941. Die Verbreitung der pelagischen Fischbrut in der südlichen Nordsee während der Frühjahrsfarten 1926–1937. *Helgoländ. wiss. Meeresunters.*, Bd. 2, Heft 2, pp. 183–225.

BRIDGER, J. P., 1956. On day and night variation in catches of fish larvae. *J. Cons. int. Explor. Mer*, Vol. 22, No. 1, pp. 42–57.

DANNEVIG, H., 1897. On the rearing of the larval and post-larval stages of the plaice and other flat-fishes. *Rep. Fish. Bd Scot.*, 15th Year (1896), pt. III, pp. 175–93.

HJORT, J., 1914. Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. *Rapp. Cons. Explor. Mer*, Vol. 20, 288 pp.

—— 1926. Fluctuations in the year classes of important food fishes. *J. Cons. int. Explor. Mer*, Vol. 1, No. 1, pp. 5–38.

JOHANSEN, A. C., 1927. On the fluctuations in the quantity of young fry among plaice and certain other species of fishes and causes of same. *Rep. Danish biol. Sta.*, Year 1927, Vol. 33, pp. 5–16.

KROGH, A., 1939. *Osmotic Regulation in Aquatic Animals*, 242 pp. Cambridge University Press.

LEBOUR, M. V., 1919. The food of young fish. *J. mar. biol. Ass. U.K.*, Vol. 12, pp. 261–324.

MARR, J. C., 1956. The 'critical period' in the early life history of marine fishes. *J. Cons. int. Explor. Mer*, Vol. 21, No. 2, pp. 160–70.

REIBISCH, J., 1902. Über den Einfluss der Temperatur auf die Entwicklung von Fischeiern. *Wiss. Meeresuntersuch., Abt. Kiel*, Bd. 6, pp. 213–31.

ROLLEFSEN, G., 1930. Observations on cod eggs. *Rapp. Cons. Explor. Mer*, Vol. 65, pp. 31–4.

SETTE, O. E., 1943. Biology of the Atlantic mackerel (*Scomber scombrus*) of North America. Part I: early life history, including the growth, drift and mortality of the egg and larval populations. *Fish. Bull., U.S.*, Vol. 50, No. 38, pp. 149–237.

SETTE, O. E. & AHLSTROM, E. H., 1948. Estimations of abundance of the eggs of the Pacific pilchard (*Sardinops caerulea*) off Southern California during 1940 and 1941. *J. mar. Res.*, Vol. 7, No. 3, pp. 511–42.

SILLIMAN, R. P., 1943. Thermal and diurnal changes in the vertical distribution of eggs and larvae of the pilchard (*Sardinops caerulea*). *J. mar. Res.*, Vol. 5, No. 2, pp. 118–30.

SIMPSON, A. C., 1951. The fecundity of the plaice. *Fish. Invest., Lond.*, Ser. 2, Vol. 17, No. 5, 27 pp.

SHELBOURNE, J. E., 1953. The feeding habits of plaice post-larvae in the Southern Bight. *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 149–59.

—— 1956. The effect of water conservation on the structure of marine fish embryos and larvae. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 275–86.

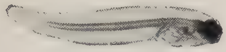
SOLEIM, P. A., 1940. Sildelarvene på vårsildefeltet. (En foreløbig meddelelse.) *Fiskeridir. Skr. Havundersøk.*, Bd. 6, No. 4, pp. 39–55.

—— 1942. Arsaker til rike og fattige årganger av sild. *Fiskeridir. Skr. Havundersøk.*, Bd. 7, No. 2, 39 pp.

SUND, O., 1924. Snow and survival of cod fry. *Nature, Lond.*, Vol. 113, No. 2831, pp. 163–4.

EXPLANATION OF PLATE I

Development stages of plaice larvae, after Simpson (left). Fig. 1. Stage 1 (6·0 mm). Fig. 2. Stage 2 (7·6 mm). Fig. 3. Stage 3 (9·2 mm). Fig. 4. Stage 4 (12·6 mm). Fig. 5. Stage 5 (12·2 mm). Condition standards of transitional plaice larvae (right). Fig. 6. Very thin (6·1 mm). Fig. 7. Thin (6·3 mm). Fig. 8. Medium (6·9 mm). Fig. 9. Robust (6·8 mm). Fig. 10. Very robust (7·5 mm).



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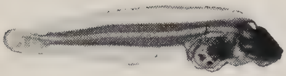

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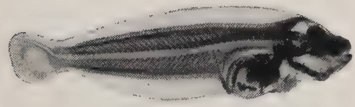

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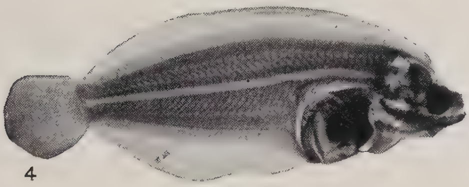

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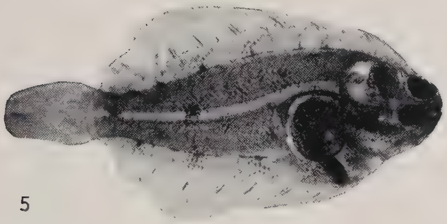

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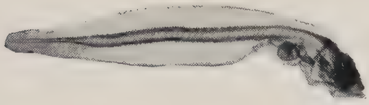

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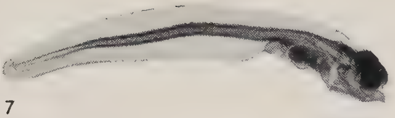

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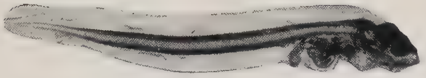

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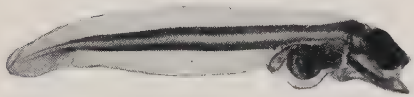

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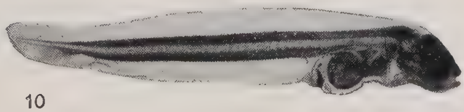

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THE RESPONSES OF *SCROBICULARIA PLANA* (DA COSTA) TO OSMOTIC PRESSURE CHANGES

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(Text-figs. 1–5)

Scrobicularia plana is an estuarine lamellibranch often numerous in intertidal, brackish muds. The animal normally lies in a burrow, 6–10 in. below the surface of the mud, and possesses long, extensible siphons through which it makes contact with the overlying water. Therefore it is probable that, as far as the salinity relations of the animal are concerned, the external medium is represented by the water above the mud.

From studies of its distribution *S. plana* is evidently tolerant of wide salinity variations in the external medium. In the Tamar estuary the species is distributed mainly in the upper half of the tidal zone down to just below mid-tide level (Spooner & Moore, 1940), and at Whitstable it is most common at mid-tide level along seaward parts of the 'bound shingle zone' (Newell, 1954). Similarly, in the extensive mud flats of the Thames estuary at Chalkwell in Essex it occurs most abundantly at mid-tide level and is completely absent from the more sandy deposits lower down the shore. This concentration of the population near mid-tide level means that the animals are not exposed to the great changes in salinity during each tidal cycle experienced by those animals living near low-water mark (Milne, 1938). However, Green (1957) in a study of a population of *S. plana* in the Gwendraeth estuary in South Wales has shown that here they are subjected to a salinity as low as two parts per thousand for a short period before being uncovered by the retreating tide.

In the Tamar, Spooner & Moore record the species along the estuary from St John's Lake, where there are 'almost marine conditions in drier parts of summer', to North Hooe, with a salinity of 20·2‰ at high water during a dry summer (Percival, 1929). In the Thames estuary the variation in salinity during that part of the tidal cycle to which an animal living near mid-tide level would be subjected is not very great. There are, however, considerable seasonal variations depending on the extent of dilution of the river by fresh water from land sources, and at Chalkwell the mid-tide salinity ranges from 25·2‰ in a wet winter to 32·4‰ in a dry summer.[1] At Whitstable the salinity does not differ greatly from that of the Channel and southern North Sea, the maximum variation at high water recorded during the years 1953–4 being from 29·58 to 34·52‰ (El-Maghraby, London Ph.D. Thesis, 1955).

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[1] All information concerning salinities at Chalkwell has been obtained from the Water Pollution Research Laboratory, and we are grateful to the Director for permission to quote unpublished figures.

These considerations make it clear that *S. plana* can tolerate almost marine conditions, salinities of about 20‰, and, for short periods at least, salinities as low as two parts per thousand.

Since *S. plana* normally experiences variations in external salinity, and since observations made during a study of rhythmic activities of this animal (Freeman, unpublished) showed that they were affected by dilution of the external medium, a study has been made of the effect of changes of external salinity on the concentration of the blood and on some aspects of the behaviour of *S. plana*.

MATERIALS AND METHODS

Animals used in these experiments were collected from the mud flats at Chalkwell in Essex. They were allowed to burrow in mud from the same situation and kept under sea-water circulation at the Plymouth Laboratory.

Blood samples were obtained from the heart, which was exposed by cutting each valve of the shell along a line between the insertions of the anterior and posterior adductor muscles, and removing that part of the shell which, freed from the restraint of the adductor muscles, was raised by the elasticity of the hinge ligament. Samples of 4–6 mg of blood for measurements of depression of the freezing-point were taken directly into capillary tubes which were immediately sealed at both ends by injection of silicone stopcock grease from a hypodermic syringe. These operations were carried out in a moist chamber saturated with respect to sea water. Depression of the freezing-point was estimated by a method similar to that of Jones (1941) and Gross (1954). The blood samples and a series of NaCl standards of appropriate concentrations, similarly sealed in uniform capillary tubes, were fastened to a glass frame with silicone stopcock grease and frozen by immersion in alcohol cooled to the temperature of solid carbon dioxide. They were immersed in a vessel of cold, 5 M-NaCl, which was kept continuously stirred, and allowed to warm slowly in a lagged box. A rate of warming of 1° C in 45 min was obtained by keeping the box in a constant temperature room at 2° C. The time taken for the complete disappearance of the crystals was noted, this being facilitated by viewing the crystals through crossed polaroids. The depression of the freezing-point of the blood samples was calculated from the curve relating melting time to the freezing-point of the standards.

Specimens used for measurement of opening rates were removed from the mud or acclimation vessel and left in air for 5 min. At the end of this time they exhibited what may be termed the normal degree of closure. In this state the shell valves are sufficiently apposed to prevent the animal protruding its foot or siphons, but the free edges of the mantle lobes are still visible between the lower edges of the shell. Here the lobes of the two sides are closely applied to form a pallial curtain that, in the absence of complete fusion, probably functionally simulates that condition. Twenty animals were placed in each experimental vessel, in which they were covered to a depth of about 2 in, and spaced widely so that the activity of any specimen would be unlikely to disturb those adjacent to it. They were observed at 5 or 10 min intervals and recorded as open if the inhalant siphon was clearly visible beyond the margin of the shell. If the foot alone was protruded or just the closed tip of the siphon was visible the animal was recorded as half open. Chapman & Newell (1956) observed that the inhalant siphon was protruded soon after an initial gaping of the shell valves and that the siphon was open at its tip during extension. The protrusion of the inhalant siphon therefore provided a convenient indication that the animal was open, in the sense of being exposed to the external medium.

Diluted sea water for all experiments was made by adding distilled water to Plymouth circulation water, which is referred to throughout as 100% sea water.

EXPERIMENTS AND RESULTS

THE RELATION BETWEEN OSMOTIC PRESSURE OF THE BLOOD AND THAT OF THE EXTERNAL MEDIUM

Although the estuarine and marine lamellibranchs that have been studied exhibit little or no osmoregulatory ability (Prosser, 1950), experiments were first carried out to determine whether *S. plana* conformed to this general picture. Animals were equilibrated at 15° C for 48 h in 100% sea water and in sea water diluted to 80, 70 and 60%. Others were equilibrated in 50% sea water for 48 h when some of them were transferred to 30% for a further 60 h, the rest remaining for the same length of time in 50%. Measurements of freezing-point depression of the blood and of the external medium are shown in Table 1.

These results show that the osmotic pressure of the blood agrees closely with that of the external medium down to dilutions of 50%, but in 30% sea water the osmotic pressure of the blood is significantly higher than that of the external medium.

The agreement of the osmotic pressure of the blood with that of the external medium means that, if, as suggested above, the external medium of the animal in its natural habitat is represented by the water above the mud, the osmotic pressure of the blood of animals taken from the mud should agree with that of the overlying water rather than that of water contained in the mud. To examine this the following experiment was carried out. Blood samples were taken from animals collected from the upper limit of their distribution at Chalkwell immediately after being uncovered by the tide, on a day (13 September 1956) when there had been a sudden decrease in flow of fresh water over Teddington weir. In Table 2 the depression of the freezing-point of the blood is compared with that of samples of water taken from above the mud 15 min before the tide receded.

These figures show that there was no significant difference between the osmotic pressure of the blood and that of the overlying water. In his study of the salinity of intertidal muds, Smith (1956) comments that although the quantitative relationship between salinity of water contained in the mud and the varying salinity of the overlying water is not known, the interstitial salinities give a picture of changes within past weeks. The immediate past history of the Chalkwell muds was of exposure to lower salinities than that given in Table 2. The records of the Thames Conservancy show that the dilution of the river by fresh water flowing over Teddington weir was less on the day when the samples were taken than on any of the preceding 10 days, and the survey of the Water Pollution Research Laboratory, which correlates freshwater flow at Teddington with chlorinity of the water along the Thames estuary, indicates that the average chlorinity at high water at Chalkwell over these preceding 10 days was about 16·5‰, and the average over the preceding 2 weeks was only 16·8‰. On the day the samples were taken the flow at Teddington was 656·2 million gallons, corresponding to a chlorinity at high

TABLE 1. RELATION BETWEEN DEPRESSION OF THE FREEZING-POINT OF THE EXTERNAL MEDIUM AND OF THE BLOOD OF *SCROBICULARIA PLANA*.

The figures in the right-hand column are calculated values of Student's *t*, applying Bessel's correction for small samples.

	No. of observations	Mean freezing-point depression (° C)	Standard deviation	*t*
100 % sea water	13	1·90	0·048	0·291
Blood	11	1·89	0·011	
80 % sea water	6	1·55	0·009	1·06
Blood	9	1·60	0·073	
70 % sea water	7	1·37	0·077	0·572
Blood	10	1·35	0·059	
60 % sea water	6	1·12	0·079	1·49
Blood	9	1·17	0·041	
50 % sea water	5	1·052	0·016	0·316
Blood	5	1·054	0·012	
30 % sea water	7	0·592	0·0104	13·6
Blood	5	0·745	0·0382	

TABLE 2. DEPRESSION OF THE FREEZING-POINT OF THE BLOOD OF *SCROBICULARIA PLANA* AT CHALKWELL

	No. of observations	Mean freezing-point depression (° C)	Standard deviation	*t*
Overlying water	9	1·756	0·025	0·51
Blood	16	1·762	0·028	

water at Chalkwell of about 17·5‰. This agrees fairly well with a calculated chlorinity of 17·85‰ for sea water with the depression of the freezing-point given in Table 2 (Robinson, 1954). The blood of the animals, therefore, agreed with the overlying water rather than with any average picture of changes within the preceding 2 weeks. Moreover, the normal situation of this deposit-feeding lamellibranch is with its siphons protruded into the overlying water, and experiments reported later in this paper show that the blood of specimens that do not protrude their siphons is almost unaffected by the salinity of the water that surrounds the shell and mantle margin. This lends support to the view stated earlier that the salinity with which the animal comes into equilibrium is that of the overlying water rather than water contained in the mud.

RATE OF EQUILIBRATION TO DILUTED SEA WATER

Although it has been shown that, except when *S. plana* is subjected to very low external salinities, its blood attains the same osmotic pressure as the external medium, no indication has been given of the time necessary for equilibration. In order to study the rate of equilibration several animals were transferred from the mud under sea-water circulation to 80 and 60% sea water at 15° C. At intervals specimens were taken at random from those in the experimental vessels and measurements made of the depression of the freezing-point of their blood. The results are shown in Fig. 1.

The time taken for equilibration was approximately 8 h in 80% sea water and 25 h in 60% sea water. Within the limits imposed by small sample size these figures give a picture of the effect on a population of *S. plana* of a sudden change in the salinity of the external medium. Most noticeable is the considerable scatter of the freezing-point of the blood of animals in 80% sea water up to 4 h, and of animals in 60% sea water up to 14 h. This scatter was interpreted as indicating that, when transferred to the dilute medium, some animals had remained closed longer than others and therefore the estimation of the freezing-point of their blood did not represent the result of a maximal exposure of their tissues to the lowered external salinity over the time stated. These readings would therefore be higher than those of animals that opened almost immediately. Furthermore, the mean of the readings for 1 and 2 h was higher for the specimens in 60% than in 80% sea water, and only very slightly lower after 4 h. The greater concentration difference to which the animals in 60% sea water were exposed should result in a much faster decrease in the tonicity of the blood. The time taken for complete equilibration should not in fact be appreciably longer than in 80% sea water (Jacobs, 1935).

In order to compare the rates of equilibration of open and closed specimens the previous experiment was repeated, but the equilibrating specimens were observed throughout the experiment and only those which had been open or closed continuously were selected for measurement of the freezing-point depression of their blood. The results are presented in Figs. 2 and 3.

These results show that the rate of equilibration of open animals is much more rapid than suggested by the previous experiment. Open animals equilibrate to 80% sea water in 4–5 h and to 60% sea water in 5–6 h. The very rapid rate of dilution of the blood over the first 15 min is particularly noticeable. In contrast the blood of closed animals changed very slowly. Those in 60% sea water, where the concentration gradient is greatest, show an average drop in their blood osmotic pressure of only 1·5% per hour, whereas those in 80% sea water show a drop of about 4% per hour.

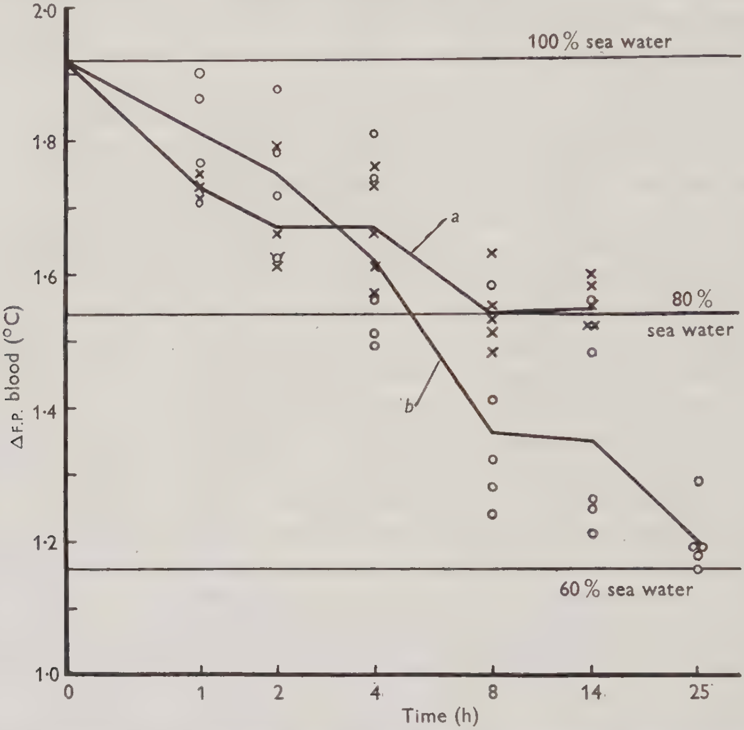

Fig. 1. Depression of the freezing-point of the blood of randomly chosen specimens of *Scrobicularia plana* after varying times of exposure to 80% (×) and 60% (o) sea water. The time scale is abbreviated by being expressed geometrically. *a*, line joining the means of the 80% readings; *b*, the same for the 60% readings.

EFFECT OF DILUTION OF THE EXTERNAL MEDIUM ON RATE OF OPENING

In the previous section it was shown that the average drop in blood osmotic pressure of a population of *S. plana* exposed to 60% sea water was slower over the first few hours than that of a population exposed to 80% sea water, whereas animals selected as having been continuously open showed a more rapid decrease in their blood osmotic pressure over this period in 60% than in 80% sea water. In view of the very small change in osmotic pressure of the blood of animals that remained closed, it seemed possible therefore that animals suddenly exposed to 60% sea water opened less rapidly than those exposed to the lesser dilution. It was indeed observed when selecting specimens that had been open or closed continuously that very few of the animals in 60% sea water opened soon after being transferred to this medium, whereas very few of those in 80% sea water remained closed throughout the experiment. The results given in the previous section also showed that specimens

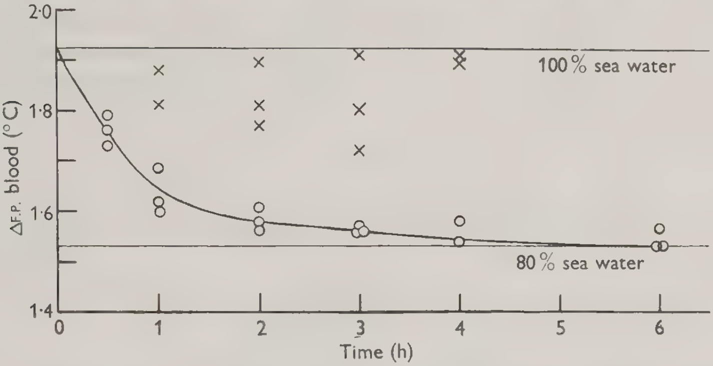

Fig. 2. Depression of the freezing-point of the blood of open and closed specimens of *Scrobicularia plana* after varying times of exposure to 80% sea water. ○, open animals; ×, closed animals.

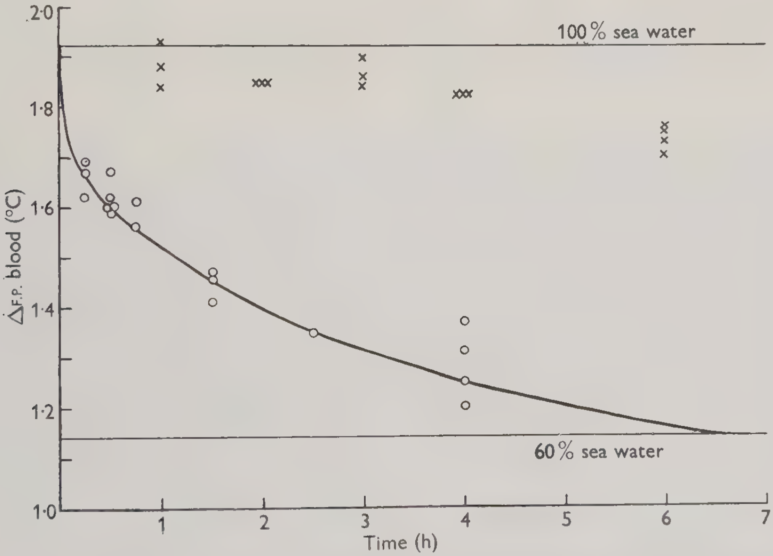

Fig. 3. Depression of the freezing-point of the blood of open and closed specimens of *Scrobicularia plana* after varying times of exposure to 60% sea water. ○, open animals; ×, closed animals.

that were selected as being closed in 60% sea water showed less change in their blood osmotic pressure than those in 80% sea water. This indication that closed animals in 60% sea water were more completely insulated from changes in external osmotic pressure than those in 80% sea water suggested that specimens remained more tightly closed in the more dilute medium. These differences might mean that 80% sea water could be regarded as a more favourable medium for *S. plana*, or that this species responds to the sudden large change in salinity from 100 to 60% sea water by remaining closed.

In order to study the response to changes in salinity the following experiments were carried out. Specimens were equilibrated for one week in running circulation water, and in vessels containing 80 and 60% sea water. They were then transferred to various dilutions of sea water and their opening rates recorded. The results are given in Table 3.

TABLE 3. OPENING RATES OF *SCROBICULARIA PLANA* EQUILIBRATED IN 100, 80 AND 60% SEA WATER, AND EXPOSED TO VARIOUS DILUTIONS OF SEA WATER.

The figures in the body of the table are the time in minutes for half of the specimens to open in each of the dilutions given in the left-hand column.

	Time of 50% opening		
Experimental concentration, % sea water	Equilibrated in 100% sea water	Equilibrated in 80% sea water	Equilibrated in 60% sea water
100	110	27	57
90	45	19	—
80	110	9	30
70	145	17	18
60	170	17	18
50	290	12	—
40	> 1 day	—	65

These results show that animals equilibrated for one week in 80 and 60% sea water open most readily when placed in that concentration to which they have been equilibrated, and that they show an increasing tendency to remain closed the greater the change in salinity to which they are exposed, whether this is an increased or decreased concentration. Those animals equilibrated in 100% sea water do not open most readily when exposed to the same concentration, and animals equilibrated in 80 and 60% sea water also open very slowly in 100% sea water. Perhaps this extreme concentration, to which these estuarine lamellibranchs are rarely exposed in their natural habitat, may be regarded as an unfavourable medium. It is also evident from Table 3 that animals equilibrated in 100% sea water opened much more slowly in all dilutions than those equilibrated in 80 or 60% sea water. This was not due to any adverse effects of a week's exposure to 100% sea water, as is shown by the following experiment. Some specimens were equilibrated in an open bowl under splashing circulation water, others were allowed to burrow in mud which was kept under circulation, and others were placed in a vessel of still 100% sea water. They were allowed to open in 100% sea water and the time for 50% of them to open was recorded. Those equilibrated in still water opened in 27 min, those under mud in 46 min, and those under splashing circulation in 120 min. It appears therefore that the very slow opening rate was not due to any difference in salinity of the water in which the animals were equilibrated, but rather to some other condition of pre-treatment. Those equilibrated in an open bowl under splashing circulation had necessarily been subjected to slight, constant vibration not experienced by those in still water or burrowed under mud, and therefore the effect of vibration was investigated in the following experiment. Specimens were equilibrated under splashing circulation and in still water and their opening rates measured in 100% sea water under conditions where some of them were exposed to constant vibration and others allowed to open under control conditions. Also, in order to determine the interaction between the effects of vibration and those of exposure to diluted sea water, the opening rate was measured in 50% sea water.

TABLE 4. THE EFFECT OF VIBRATION AND EQUILIBRATION CONDITIONS ON THE OPENING OF *SCROBICULARIA PLANA*

All specimens equilibrated in 100% sea water. Opening rates expressed as time in minutes for 50% opening.

	Equilibrated under splashing circulation		Equilibrated in still water.
	Experimental concentration 100%	Experimental concentration 50%	Experimental concentration 100%
Control	175	250	53
Vibrated	55	129	17

Table 4 shows that all the vibrated animals opened more rapidly than those not vibrated. It also shows that the animals equilibrated in still water opened more rapidly than those equilibrated under splashing circulation under both control and vibrated conditions. The vibration acts as a stimulus to open which is superimposed on the control opening rates. Of the animals equilibrated under splashing circulation, those exposed to 100% sea water opened more rapidly than those in 50% sea water, emphasizing the effect of concentration of the medium in influencing opening rate, but those vibrated in 50% sea water opened more rapidly than those not vibrated in 100% sea water. Thus, under these conditions, the effect of concentration of the medium can be completely over-ridden by the effect of vibration.

EFFECT OF DILUTION OF THE EXTERNAL MEDIUM ON OPEN ANIMALS

In previous experiments the effect of changing the concentration of the external medium has been studied only on closed animals, but it is probable that, when burrowed in the mud in its natural habitat, *S. plana* would be exposed to a changed salinity only when its siphons were protruding into the overlying water. To determine the response of animals with extended siphons to changed salinity, two groups of animals were allowed to open in 100% sea water and when a convenient number were open the water was siphoned out and replaced by 50% sea water in one bowl and by 100% sea water in the other. The effect of this change on the number of animals open is shown in Fig. 4.

This shows that the process of replacing the water did not in itself affect the number of animals open, but the sudden change from 100 to 50% sea water caused more than half of the animals to close.

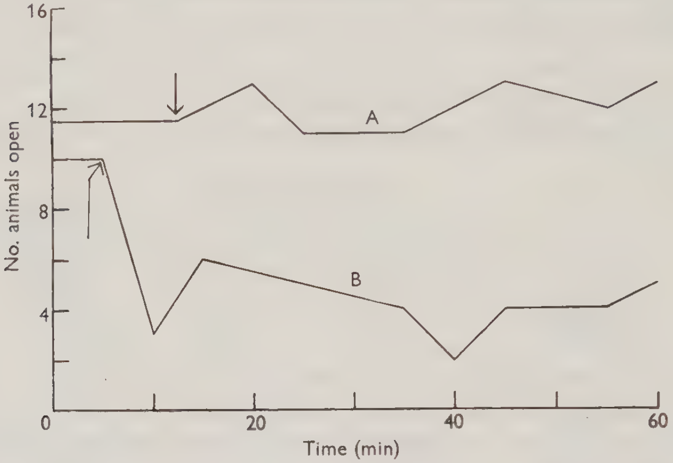

Fig. 4. Effect of dilution of the medium on numbers of open *Scrobicularia plana*. 100% sea water replaced by (A) 100% sea water, (B) 50% sea water, at points marked by arrows.

In this, as in previous experiments where the effect of changed concentration of the external medium has been studied, the medium has differed from that to which the animal was equilibrated both in concentration of individual ions and also in total osmotic pressure. To test which of these factors was significant in influencing the behaviour of *S. plana*, closed specimens were exposed to 100% sea water, 50% sea water, and sea water to which was added an equal volume of 0·9 M sucrose solution. This latter medium is equivalent to 50% sea water in ionic concentration and to 100% sea water in total osmotic pressure. The opening rates are compared in Fig. 5.

These results show that animals in the 50% sea water containing 0·45 M sucrose behave almost identically with those in 100% sea water and very differently from those in 50% sea water. The factor that tends to keep *S. plana* closed when suddenly exposed to media of different concentration is evidently an alteration in total osmotic pressure.

Some measurements were also made of the depression of the freezing-point of the blood of animals that had been exposed to the 50 % sea water containing 0·45 M sucrose. Those that had opened showed signs of very considerable dehydration and with many it was found impossible to obtain enough blood for estimation. Samples from three animals each gave a depression of the freezing-point of 1·83° C, as compared with 1·91 and 1·92 for the blood of the only two specimens that did not open at all during the experiment. Presumably under these conditions the initial tendency is for the blood to lose ions to the medium, resulting in a decreased internal osmotic pressure, with a consequent outward movement of water. The blood might therefore be expected at any one time to show a slightly lower osmotic pressure than the external medium. It was noted that severely dehydrated specimens returned to sea water recovered to an apparently normal condition, although there would be no osmotic gradient tending to bring about a re-entry of water.

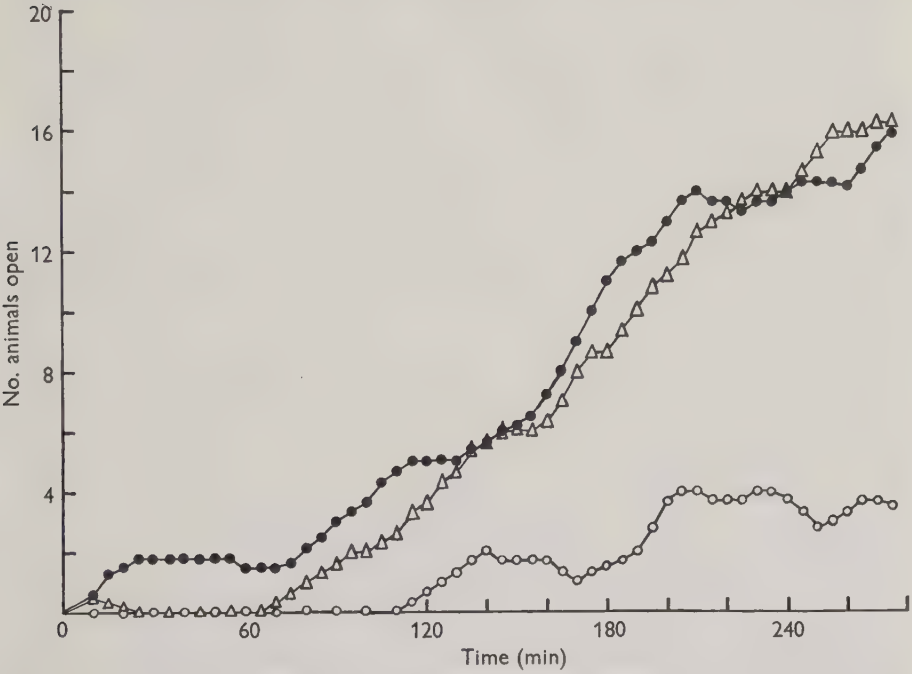

Fig. 5. The opening rates of *Scrobicularia plana* in sea water, 50 % sea water, and 50 % sea water made isotonic with an equal volume of 0·9 M sucrose solution. ●—●, 100 % sea water; △—△, 50 % sea water containing 0·45 M sucrose; ○—○, 50 % sea water. (The numbers plotted are the moving average of three readings.)

DISCUSSION

Scrobicularia plana is similar to other estuarine and marine lamellibranchs that have been studied in showing no osmo-regulatory ability, except at very low external salinities. However, it reacts to extreme changes in external salinity by remaining closed, or, if open, by retracting its siphons, and thus protects its blood from extreme changes in concentration. These responses are to the stimulus of an alteration in total osmotic pressure of the external medium, which the animal can assess even while in the closed condition.

Variations in behaviour in response to varying external salinities have been observed in some other lamellibranchs. Maloeuf (1938) observed the maintained closure of *Mytilus edulis* in distilled water and found it necessary to wedge specimens open to expose the tissues to the external medium, and Morton, Boney & Corner (1957), working on the small lamellibranch *Lasaea rubra*, noted a greater number open after 5 min in sea water as compared with dilute and concentrated sea water. It is not known whether these responses of *Mytilus edulis* and *Lasaea rubra* are directly related to the appreciation of osmotic pressure differences, nor to what extent they are accompanied by internal physiological changes.

In groups of animals other than lamellibranchs, changes in behaviour have been correlated with physiological changes. Hughes & Kerkut (1956) have shown that there is an increase in the spontaneous activity of the isolated pedal ganglion of the slug *Agriolimax reticulatus* on dilution of the medium in which the ganglion is immersed. They argue that, in an animal with an open blood system such as a mollusc, this bathing medium corresponds to the blood or haemolymph of an intact animal. They correlate this increased electrical activity of the nervous system with the changes of behaviour of the slug during conditions of hydration, when its blood is diluted, and conclude that the increased rate of discharge of the ganglion is probably not related to any particular change in behaviour but represents a difference in the 'tonus' or 'vigilance' of the central nervous system against which reflex activity will be expressed. In an extension of this work, Kerkut & Taylor (1956) have shown that the effective change to which the ganglion responds is one of osmotic pressure, and that the limiting rate producing an increase in ganglionic activity is a change of 1% in 4 min. They discuss these results in the light of other osmoreceptors known, particularly in mammals where there is a similar phenomenon of part of the central nervous system being sensitive to osmotic pressure changes in the blood.

The situation in *Scrobicularia plana* differs in one important respect from that in the slug. On exposure to external media of lowered osmotic pressure *S. plana* shows a greater reluctance to open than in sea water, there being a greater tendency to remain closed the greater the decrease in osmotic pressure. These changes in behaviour are accompanied by a much smaller rate of change in blood concentration than that found to be limiting in the slug. If the increased 'vigilance' of the central nervous system, as a result of dilution of the blood, is responsible for these changes in behaviour in *S. plana*, the parts of the nervous system concerned must be at least eight times as sensitive to these changes as those of the slug.

It seems more likely that in a lamellibranch such as *S. plana*, able to protect itself from the effects of gross variations in the external medium by closing its shell, there is some part of the animal still retaining a degree of contact with the external medium which is able to appreciate the sudden changes in osmotic pressure to which the animal has been shown to respond. It has already been observed that the free edges of the mantle lobes do retain such contact, and in view of the development of sensory structures such as tentacles and eyes in this region in certain other lamellibranchs, it seems possible that some osmoreceptive function may be located there in *S. plana*.

Although the effect of osmotic pressure on the behaviour of *S. plana* has been stressed in this paper, it is by no means the only factor influencing the opening of lamellibranchs. Woortmann (1926) has shown the effect of changes in oxygen and carbon dioxide concentration on the opening of *Mytilus edulis*, and it has been shown in this paper that previous acclimation conditions affect the opening rate of *Scrobicularia plana* in all concentrations of sea water, and that the adverse effect of a large change in salinity can be completely overridden by slight vibration.

The experiments described in this paper give some indication of the effect of salinity changes on *S. plana* in its normal environment. In estuaries such as those of the Tamar and Thames, the species will be equilibrated to the osmotic pressure of the overlying water. This will change with seasonal variations in the fresh-water flow into the estuary and will be accompanied by corresponding changes in the blood. Members of the species exposed to sudden changes, such as those in the Gwendraeth estuary, will, if open, tend to close, but if they remain open they will experience a very rapid drop in their blood osmotic pressure. If already closed, and in a situation where they are exposed to the lowered salinity, they will tend to remain closed, with their blood almost unaffected.

Estuarine lamellibranchs occurring below low-water mark may experience longer immersion in low salinity water than *S. plana*, which, as Spooner & Moore remark, appears intolerant of permanent immersion. Percival records extensive beds of *Mytilus edulis* off Neille Point in the River Tamar lying in 12–18 in. of water at low tide where the salinity may go down to 2·8‰ in wet weather. It would be interesting to know if similar considerations to those discussed for *Scrobicularia plana* apply here also.

All the experiments reported in this paper were carried out at the Plymouth Laboratory and we are grateful to the Director and his staff for many kindnesses shown during the course of this work. We are particularly indebted to Dr E. D. S. Corner for the benefit of much helpful advice and useful criticism. We also wish to thank Dr G. E. Newell and Dr G. Chapman for reading the manuscript.

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SUMMARY

The osmotic pressure of the blood of *Scrobicularia plana* has been measured when the animal is exposed to diluted sea water, and observations made on the behaviour of the animal when exposed to solutions of different osmotic pressure.

The blood osmotic pressure shows no significant difference to that of the external medium except in very low salinities. The external medium with which an animal in its natural habitat comes into equilibrium is represented by the water above the mud rather than the water contained in the mud. Open animals equilibrate to 80% sea water in 4–5 h and to 60% sea water in 5–6 h. The osmotic pressure of the blood of animals that remain closed in dilute media is decreased by as little as 1·5% per hour.

Closed animals equilibrated in concentrations less than 100% sea water open most readily when exposed to that concentration to which they are equilibrated. If exposed to changed concentrations they tend to remain closed, the greater the change in concentration the greater the tendency to remain closed. Open animals exposed to changed concentrations tend to close. The effective stimulus to which the animal responds is the total osmotic pressure of the medium. The opening rate is affected by the conditions under which the animals have been equilibrated, and also by slight vibration.

These results are discussed with reference to other lamellibranchs and to other animals that respond to osmotic pressure changes.

REFERENCES

Chapman, G. & Newell, G. E., 1956. The rôle of the body fluid in the movement of soft-bodied invertebrates. II. The extension of the siphons of *Mya arenaria* L. and *Scrobicularia plana* (da Costa). *Proc. roy. Soc.* B, Vol. 145, pp. 564–80.

Green, J., 1957. The growth of *Scrobicularia plana* (da Costa) in the Gwendraeth estuary. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 41–7.

Gross, W. J., 1954. Osmotic responses in the sipunculid *Dendrostomum zostericolum*. *J. exp. Biol.*, Vol. 31, pp. 402–23.

Hughes, G. M. & Kerkut, G. A., 1956. Electrical activity in a slug ganglion in relation to the concentration of Locke solution. *J. exp. Biol.*, Vol. 33, pp. 282–94.

Jacobs, M. H., 1935. Diffusion processes. *Ergebn. Biol.*, Bd. 12, pp. 1–160.

Jones, L. L., 1941. Osmotic regulation in several crabs of the Pacific coast of North America. *J. cell. comp. Physiol.*, Vol. 18, pp. 79–92.

Kerkut, G. A. & Taylor, B. J. R., 1956. The sensitivity of the pedal ganglion of the slug to osmotic pressure changes. *J. exp. Biol.*, Vol. 33, pp. 493–501.

MALOEUF, N. S. R., 1938. Studies on the respiration (and osmoregulation) of animals. I. Aquatic animals without an oxygen transporter in the internal medium. *Z. vergl. Physiol.*, Bd. 25, pp. 1–28.

MILNE, A., 1938. The ecology of the Tamar estuary. III. Salinity and temperature conditions in the lower estuary. *J. mar. biol. Ass. U.K.*, Vol. 22, pp. 68–87.

MORTON, J. E., BONEY, A. D. & CORNER, E. D. S., 1957. The adaptations of *Lasaea rubra* (Montagu), a small intertidal lamellibranch. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 383–405.

NEWELL, G. E., 1954. The marine fauna of Whitstable. *Ann. Mag. nat. Hist.*, Ser. 12, Vol. 7, pp. 321–50.

PERCIVAL, E., 1929. A report on the fauna of the estuaries of the River Tamar and River Lynher. *J. mar. biol. Ass. U.K.*, Vol. 16, pp. 81–108.

PROSSER, C. L. (ed.), 1950. *Comparative Animal Physiology*. Philadelphia and London.

ROBINSON, R. A., 1954. The vapour pressure and osmotic equivalence of seawater. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 449–55.

SMITH, R. I., 1956. The ecology of the Tamar estuary. VII. Observations on the interstitial salinity of intertidal muds in the estuarine habitat of *Nereis diversicolor*. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 81–104.

SPOONER, G. M. & MOORE, H. B., 1940. The ecology of the Tamar estuary. VI. An account of the macrofauna of the intertidal muds. *J. mar. biol. Ass. U.K.*, Vol. 24, pp. 283–330.

WOORTMANN, K.-D., 1926. Beiträge zur Nervenphysiologie von *Mytilus edulis*. *Z. vergl. Physiol.*, Bd. 4, pp. 488–527.

J. mar. biol. Ass. U.K. (1957) 36, 569–592
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LERNAEOCERA OBTUSA N.SP., A HITHERTO UNDESCRIBED PARASITE OF THE HADDOCK (*GADUS AEGLEFINUS* L.)

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(Text-figs. 1–6)

The present genus *Lernaeocera* comprises seven species. Four of them are known to occur on the western European seaboard. They are: *L. branchialis* (Linnaeus, 1767) *L. lusci* Basset-Smith, 1896, *L. minuta* T. Scott, 1900, and *L. brevicollis* Schuurmans Stekhoven, 1935. The three non-European species have been based on isolated specimens from Chile (*L. rigida* Krøyer, 1863), New Zealand (*L. lotellae* Thomson, 1889) and Antarctica (*L. godfroyi* Quidor, 1912). All are parasitic on fish.

The final hosts infested by the members of this genus can be divided into two groups: the 'inshore' group, comprising those fishes which visit the coastal waters at some stage of their lives and which are exposed to infestation by a parasite reproducing in that zone only; and the 'offshore' group, comprising those which do not visit the coastal waters at any stage and which can be infested only by a parasite with a totally offshore life cycle. One of the common hosts, the Haddock, *Gadus aeglefinus* L., can be included in both groups, as its small coastal populations can be treated, for our purposes, separately from its main offshore stocks. Out of 31 recorded hosts of the European species 27 belong to the inshore group. All the common final hosts, with the exception of the offshore haddock, and all the known intermediate hosts also belong to this group. The present genus *Lernaeocera* might be regarded, therefore, as essentially coastal in character.

L. branchialis, as it has been presented in the previous literature, stands out as conspicuously unusual, when considered against the background of its own genus. It appears to be the only species with the common hosts both in the inshore and the offshore group. In the former it is known to infect commonly such hosts as the Cod (*Gadus callarias* L.) and the Whiting (*G. merlangus* L.), and in the latter—where no intermediate host has been recorded—the haddock. Of all the other species of the genus only one, *Lernaeocera lusci*, has been recorded from offshore hosts but none of these can be regarded as common. Both *L. lusci* and *Lernaeocera* found on haddock resemble each other in the shape of the body, although the latter attains larger sizes. Both also differ in shape from the *L. branchialis* found on the hosts of the inshore group.

In his study of *L. branchialis* the attention of the present author was drawn to this difference between this species and the remaining members of the genus, and also to the differences within the species itself. It seemed that these differences could be explained if it could be proved that the name *L. branchialis* has been used hitherto to cover two distinct species, one from the whiting and the cod and the other from the haddock. Closer investigation has shown that this, indeed, is so. In this paper the existence of the new species is demonstrated. The evidence is to be found in the geographical distribution, specificity and morphology of the adult male and female and in the mode of attachment to the final host.

DISTRIBUTION

Parasites of the genus *Lernaeocera* can become endemic only in the areas inhabited by a suitable intermediate host and their distribution cannot be considered without reference to their intermediate hosts. These are known only for *L. branchialis*, the common intermediate host of which appears to be the Flounder, *Platichthys flesus* (L.) (A. Scott, 1901; Sproston, 1941). Gouillart (1937) and Stekhoven (1936) also list Plaice, *Pleuronectes platessa* L., Gouillart (1937) lists Turbot, *Scophthalmus maximus* (L.), while Oorde de Lint & Stekhoven (1936) mention 'other pleuronectids' without naming any. Shulman & Shulman-Albova (1953) found that the Arctic Flounder, *Liopsetta glacialis* (Pallas) and the Lumpsucker, *Cyclopterus lumpus* L., were—beside the flounder—common intermediate hosts of *L. branchialis* in the White Sea, and they reached the conclusion that the reproductive cycle of this parasite is exclusively coastal in character. Gouillart (1937) also mentions *C. lumpus*, but regards the larvae found on it as being specifically distinct from *L. branchialis*.

The distribution of the fish infested exclusively in the coastal area can be expected to present a gradient with the incidence decreasing as the distance from the shore increases. Such a gradient has been found by Sproston & Hartley (1941) for Whiting and Pollack, *Gadus pollachius* L., infested with *L. branchialis*, and by Templeman (1953) for the cod of the north-west Atlantic infested with the same parasite. The present author studied the distribution of the infested whiting and haddock by examining the commercial catches landed in the Aberdeen Fish Market and the catches made by the research vessels of the Marine Laboratory, Aberdeen. Over 2000 whiting and 30,000 haddock were examined. It became apparent that the two species differ remarkably in the distribution of their infested individuals. The incidence of *Lernaeocera* in whiting fully conformed with the expected pattern. Table 1 shows the results of the examination of 407 whiting in August 1956. The incidence of infestation decreased with the distance from the shore and also markedly with the size of the fish. Examination of 474 whiting in the

Firth of Forth in November 1956 showed that the incidence of *Lernaeocera* tended to decrease eastwards, i.e. towards the mouth of the Firth, and to cease in the waters within the area 10 miles west of May Island. Of the 1300 whiting caught commercially only three were found to be infested. All three were taken a considerable distance from the shore, but constituted such an insignificant proportion of the fish examined that they can be disregarded in any consideration of the distribution.

Infested cod can be found over the entire area of the northern North Sea, but only in small numbers. Of 832 cod examined during the second half of 1955 only 31, or 3·7% were found to be infested. No distinct pattern of distribution has emerged, but it seemed possible that the fishes were infested inshore and migrated outwards. Of the 31 infested individuals only four carried parasites which were not fully mature and all of these were caught no farther than 18 miles from the shore.

The distribution of the infested haddock bore no relation to the distance from the shore at all; neither did the state of the maturity of the parasites. All stages of development, including the youngest post-larval stage, the 'penella', were found at varying distances; the duration of the latter stage is estimated by Capart (1948) to be about 10–15 days. It is difficult to imagine that haddock could frequently be infested near the coast and cover a distance of 100 miles or more in the period of time during which the parasite will remain in this stage of development. The 'penella' stage was found on haddock of all sizes and ages and was not restricted to the younger and smaller individuals which one associates with the inshore waters.

These facts suggested that an intermediate host must exist in the offshore zone and that only a parasite with the reproductive cycle independent of the inshore region can be responsible for the distribution shown by the *Lernaeocera*-infested haddock. For this reason, in the spring of 1956, a search was made for an intermediate host which could account for the completion of the life cycle of the *Lernaeocera* affecting offshore haddock. Six common species of flatfishes were investigated, from numerous localities both in- and offshore (Fig. 1). The results are summarized in Table 2. Over 95% of the investigated Lemon Sole, *Microstomus kitt* (Walbaum), were infested with larval *Lernaeocera*. No other species was found to be infested. Especially noteworthy is the absence of the larvae from plaice, which is regarded by Stekhoven (1936) as one of the common intermediate hosts of *L. branchialis*. No locality shown in Fig. 1 was found to be free of *Lernaeocera*. There is no previous record of the occurrence of *Lernaeocera* in the lemon sole.

As a further check, the distribution of infested haddock was compared with that of lemon sole in the North Sea as determined by Rae (1939). The comparison made it clear that the incidence of the infestation of haddock was fairly closely related to the abundance of the lemon sole in this region.

The problem emerged: why is the offshore haddock infested with

TABLE 1. DISTRIBUTION OF INFESTED WHITING, AS SHOWN BY THE FISH EXAMINED IN AUGUST 1956 BY F.R.S. *EXPLORER*

Date	Locality	Position	Distance from shore (miles)	Depth (fm.)	Size (cm.)	Examined	Infested	Percentage infested
12. viii.	Off Cruden Scaurs	57° 20½′ N., 1° 48′ W.	*ca.* 4	35	21–23	70	6	8·6
12. viii.	Edge of Buchan Deeps	57° 24′ N., 1° 28′ W.	*ca.* 14	42	<25	115	5	4·3
					>25	222	1	0·4
					Total	337	6	1·8

TABLE 2. LIST OF FLATFISHES EXAMINED FOR THE INCIDENCE OF LARVAL *LERNAEOCERA*

Species	Examined	Infested	Percentage infested
Lemon sole, *Microstomus kitt* (Walbaum)	222	211	95·0
Plaice, *Pleuronectes platessa* L.	59	—	—
Witch, *Glyptocephalus cynoglossus* (L.)	60	—	—
Megrim, *Lepidorhombus whiff-iagonis* (Walbaum)	96	—	—
Dab, *Limanda limanda* (L.)	219	—	—
Long rough dab, *Drepanopsetta platessoides* Gill	289	—	—

Lernaeocera, while the offshore whiting is free from infestation? The differences in behaviour and the habitat between the two species provide no answer. While they could explain lower incidence of infestation of whiting, they cannot account for its total absence in this fish. It is apparent therefore that there is only one other possible explanation—the parasite which infests haddock in the open sea is specifically distinct from the one which lives inshore and infests other gadoid species.

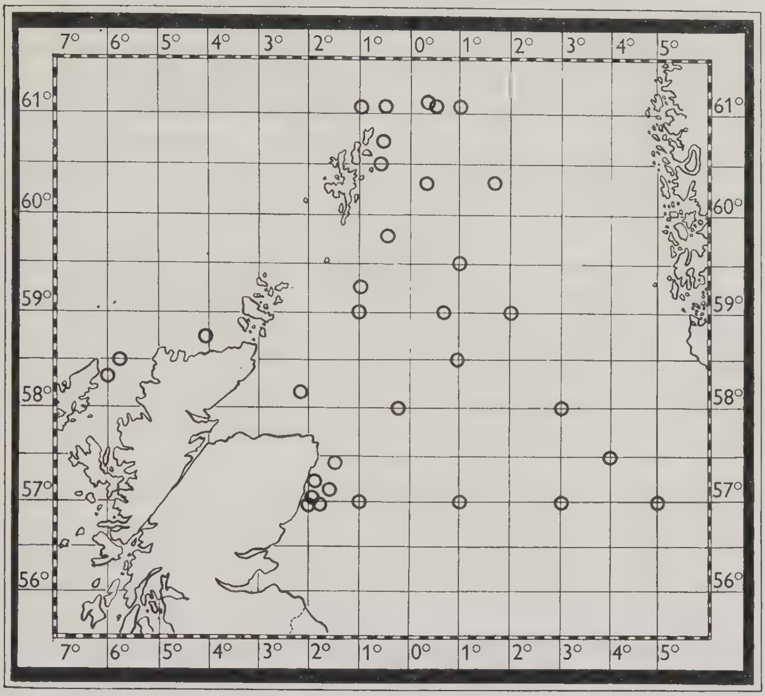

Fig. 1. Localities in which lemon sole infested with *Lernaeocera* have been found.

Investigations in Faröe waters in October 1955 showed, quite surprisingly, the complete absence of *Lernaeocera* from the haddock of that area. The numbers of fish examined on that occasion were 2450 haddock, 477 whiting and 155 cod.

Only the cod was found to be infested by *Lernaeocera* (6 individuals, 5% of the total examined). One of the infested cod carried a 'penella' stage of the parasite. Three were too young to have been migrants from elsewhere. Similar results have been obtained in October 1956, when there were examined 1434 haddock, 123 whiting and 174 cod.

This time 8% of the cod examined were found to be infested, and some of the 15 infested individuals were again too young to be anything but local fish. Only one haddock was infested with a 15 mm long 'penella' stage.

These results show that *Lernaeocera* is endemic in the Faröe waters. It has presumably spread into that area from the south, possibly carried by cod, and has become established there in presence of an intermediate host. Haddock, on the other hand, do not migrate to Faröe from the North Sea. Both flounder and lemon sole are endemic in Faröe.

The patterns of the distribution of *Lernaeocera* in the North Sea showed that the parasite of cod and whiting could be classed together and separately from that of the haddock. The absence of *Lernaeocera* from the Faröese whiting was understandable, since all the examined whiting fell in the offshore category. There was, however, no reason for the absence of the parasite from the haddock, unless it was unable to infest it. Specific incompatibility provides the only possible explanation.

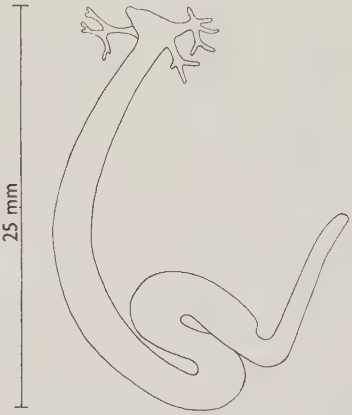

Fig. 2. Abnormal specimen of *L. branchialis* from Iceland haddock.

Investigations were also carried out in Iceland waters. No whiting were caught, but a sample of 250 cod and 299 haddock was examined for *Lernaeocera*. Of these, 23 cod of widely separated areas were infested, but only one haddock carried *Lernaeocera*. The parasite on the haddock was not normally developed (Fig. 2). It was implanted in the branchial arch, all three antlers were present and the length of the body, from the head to the genital flexure, was 25 mm. The widest diameter of the body was no more than $1\frac{1}{2}$ times that of the neck. No egg-strings were present. The general impression was of the retention of juvenile characteristics with simultaneous increase in size, a feature not uncommon in parasites attached to a 'wrong' host.

The geographic distribution of *Lernaeocera* of the cod and the whiting on the one hand and of the haddock on the other suggests that the existence of two species covered until now by the specific name *branchialis* is at least possible.

It should also be mentioned that no records of the infestation of haddock with *Lernaeocera* in either Faröe or Iceland were found by the author in literature.

MORPHOLOGY OF THE ADULT FEMALE

The variations and irregularities of the body shape of *L. branchialis* have prompted some workers (Stekhoven, 1936; Dollfus, 1953) to regard it as a rather unreliable specific feature. It has been felt that the shape of the body might be largely determined by the species and the size of the host. The volume and the shape of the subopercular space, depending on the species of the host, and the abundance of food, depending on its size, were regarded as the main factors determining the shape and the size of the parasite's body.

A logical chain of causes and effects is, smaller host—less food for the parasite—poorer growth—smaller parasite. When, however, it is followed a step further, a conclusion will be reached that the very fact of the parasite being smaller must restore the balance between the size of the parasite and the space available for it. Also, the subopercular spaces of related hosts of equal sizes do not differ sufficiently to produce any marked differences in the shape of the body of the parasite. Consideration of hosts of the same species shows, for example, that although the parasites of small and of large whiting differ in size, and usually also in age, no differences can be observed between the shapes of their bodies.

It must be remembered that the parasite growing in the subopercular space is not enclosed by rigid and immovable walls. The opercula perform constant movements, the effect of which can be compared rather to a series of pushes than a steady pressure. The body bends in the neck region as the result of these pushes and assumes the direction of growth parallel to the longitudinal axis of the body of the host, i.e. in the direction in which the maximum of the available space can be found. Very often a perfectly developed and not at all misshapen parasite is found protruding from under the operculum of the host. The obviously cramped conditions of growth do not seem to have affected the shape of its body. The occurrence of abnormalities must be expected, but they can be taken into account and do not affect the main argument. The existence of the genetical range of variability in shape must also be considered.

The following details of the body shape have been used as specific characters in *Lernaeocera* by the earlier workers. (i) The shape of the antlers (cross-section: T. & A. Scott, 1913; the mode of branching: Stekhoven, 1936). (ii) The structure of the cephalothoracic appendages (2nd antennae, 2nd maxillae: Stekhoven, 1936). (iii) The shape of the trunk (various authors).

The shape of the antlers is a very unreliable character and has been regarded as such even by those who applied it as an auxiliary feature in the diagnosis of the species. Hardly any two parasites can be found with the identical environmental conditions for the growth of the antlers. The difference in the cross-section of the antlers can be used only to distinguish between *L. lusci* (cross-section oval) and all the other species of the genus (cross-section round).

The structure of cephalothoracic appendages, although it has been suggested as a possible distinguishing characteristic, must be used very cautiously in diagnosis of the species, since the existence of two or more species with the identical structure of these appendages is not impossible.

The shape of the antlers was not helpful in distinguishing *Lernaeocera* found on whiting and on haddock. The structure of the cephalic appendages in the parasites found on these species showed no appreciable differences. Attention was then focused on the shape of the main trunk of the body, which has never been a subject of careful and detailed study. Most of the existing descriptions of the shape of the trunk can be applied to more than one species of *Lernaeocera*. It must be remembered also that most of the descriptive work on the structure of *L. branchialis* is based on specimens found on cod and whiting. Very few, if any, *Lernaeocera* from haddock were thoroughly examined. Of 32 specimens examined by Wilson (1917) none was from haddock, while Stekhoven (1936) had only two parasites from that host from the total of 143 specimens at his disposal.

In the course of this work more than 4000 fully developed specimens of *Lernaeocera* from haddock have been examined. It has not been possible to examine them all thoroughly, but observations have been made on the average shape of the parasite's body. It became obvious that a definite difference existed between the average shapes of *Lernaeocera* from whiting and from haddock. With one exception (Blainville, 1822) the whiting *Lernaeocera* corresponds with all the illustrations made by earlier workers, but the haddock *Lernaeocera* differs from them in a number of easily observable points. When the study of distribution suggested the possibility of the existence of two species, these apparent morphological differences were investigated more closely on the samples of over 60 specimens of mature *Lernaeocera* from both whiting and haddock. The following details of structure were examined: (i) the differences between the angle of flexure of genital segments; (ii) the relations between the thickness of the neck and the main trunk; (ii) the differences of the body shape in the neck and trunk junction area.

Genital flexure. The angles of flexure of the genital segment were measured with a Starret protractor. The results obtained are graphically presented in Fig. 3, which shows a definite difference between the two parasites. More details are given in Table 3. The parasite found on whiting had usually a sharply flexed abdomen. Out of 63 individuals examined only three had the angle of flexure wider than 90° and the average value was 66°. The angle of flexure in the haddock parasite was decidedly more obtuse and averaged 99°, with values up to 140° being found. The ranges overlapped to the extent of 18·8 %.

A sample of *Lernaeocera* collected from Iceland cod in September 1956 was also examined. Although there were somewhat more specimens over 90° than in the parasite of whiting, the cod *Lernaeocera* certainly resembled that from the whiting more strongly than it did that from haddock. Its mean value for the genital flexure was only 4° larger than that of the whiting's parasite, whereas in the haddock *Lernaeocera* it was as much as 33° larger. This feature, as the patterns of distribution and the mode of infestation, tends to draw the dividing line between the parasites of cod and whiting on one side and that of the haddock on the other.

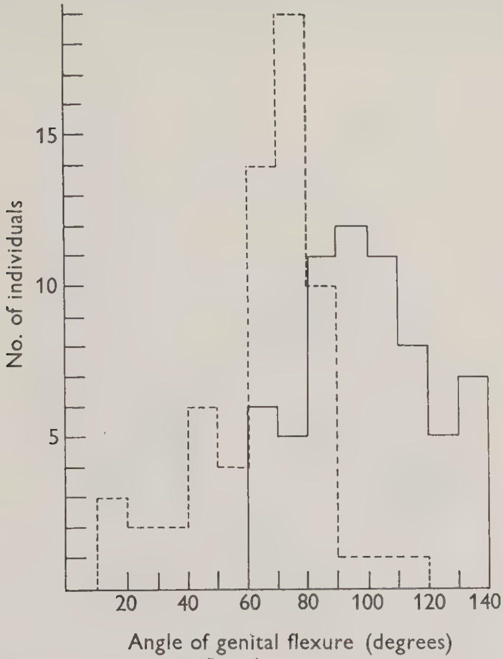

Fig. 3. Distribution of values for angle of genital flexure of *Lernaeocera*. Solid line, parasite of haddock; interrupted line, parasite of whiting.

TABLE 3. THE ANGLES OF GENITAL FLEXURES OF *LERNAEOCERA* FROM DIFFERENT HOSTS

Host species	No. of parasites examined	Range	Mean	More than 90°	Less than 90°
Haddock	65	61° 30′–139° 45′	99°	44	21
Whiting	63	17° 45′–110° 40′	66°	3	60
Cod (Iceland)	25	21° 10′–125° 10′	70°	6	19

The main trunk of the average haddock *Lernaeocera* bears very close resemblance to that of *L. lusci*, and it would not be surprising if at least some of the records of the latter species from haddock and other hosts of the offshore group proved attributable to the smaller individuals of normal haddock *Lernaeocera*. This surmise removes the difficulty of assuming an 'open sea' reproductive cycle for *L. lusci* which is otherwise a coastal species.

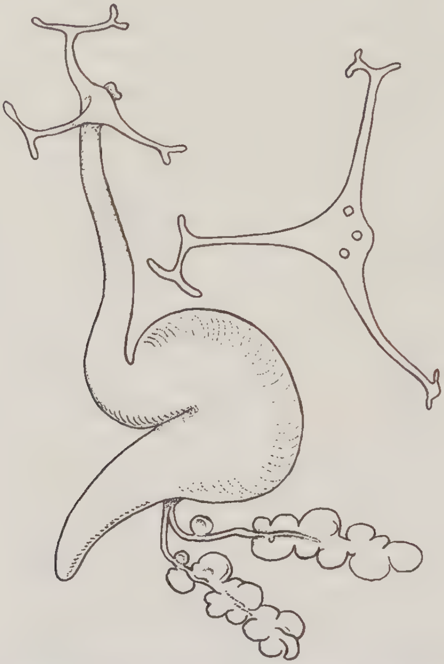

Fig. 4. (Redrawn from Blainville.) *Lernaeocera* showing typical features of the haddock parasite.

It is interesting to note that Blainville (1822), who states in his paper that *L. branchialis* occurs on *Gadus aeglefinus* and *G. barbatus*,[1] illustrated in his drawing of that parasite a body of such typically 'haddock type' that no doubt can be held as to which species was the host of that particular specimen (Fig. 4).

Relation of neck and trunk diameters. Another pronounced difference between the parasites is the relation between the diameters of the neck and trunk. There can be little doubt as to the validity of this feature in the specific diagnosis. The length of the neck may well be dependent on environmental influences, but these can in no way account for marked differences in its thickness and its relationship to the thickness of the main trunk.

[1] According the Günther (1862) the name *Gadus barbatus* is applied to (*a*) younger stage of European Cod, (*b*) Greenland species, *G. ogac*, (*c*) *G. luscus*.

A quantitative check of these differences has been made on 40 individuals from both whiting and haddock and the results are summarized below:

	Range	Mean
Haddock	0·35–0·72	0·50
Whiting	0·23–0·44	0·31

The measurements show that the neck of the haddock parasite is relatively much thicker than that of the whiting, although here again the ratios exhibit a range of variation and some overlap.

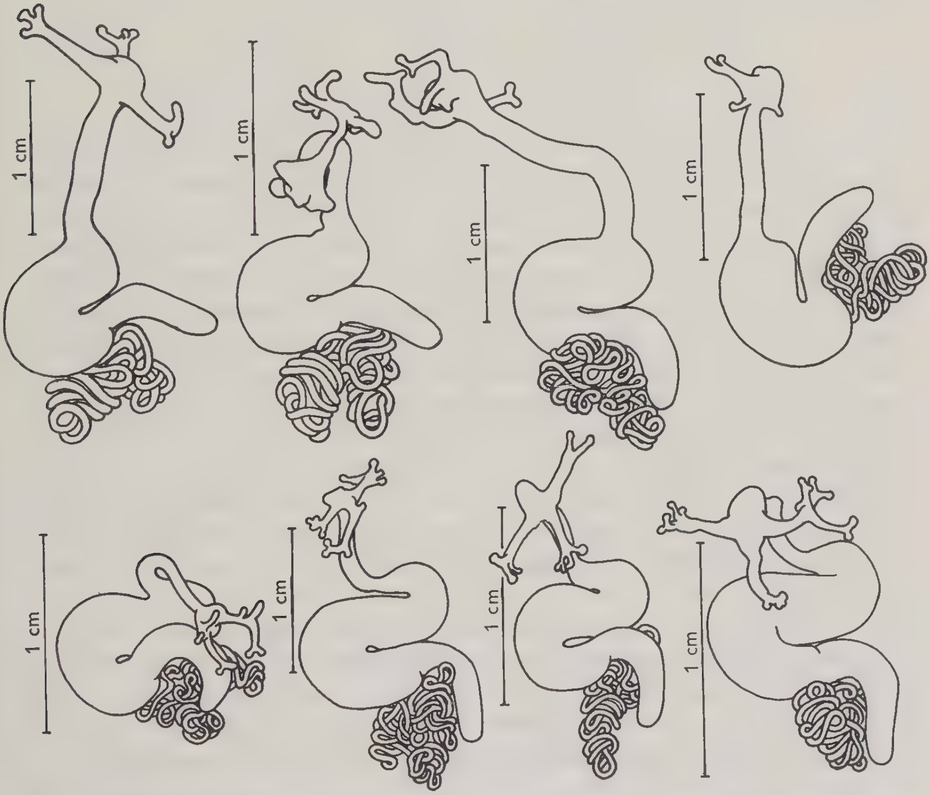

Fig. 5. Morphological differences between *L. branchialis* and *L. obtusa*. Upper, *L. obtusa* from haddock; lower, *L. branchialis* from whiting. Not to scale, but a line indicating 1 cm is drawn beside each specimen.

Shape of neck and trunk junction. Fig. 5 illustrates the third feature distinguishing between the *Lernaeocera* of whiting and haddock. It will be observed that the way in which the neck merges into the trunk is different in both animals. In the parasite of whiting the transition is sudden and well marked. The thin neck abruptly dilates into the trunk with the formation of a prominent bulge at the point of junction of the two parts. The prominence of the bulge is accentuated by the thinness of the neck. In the parasite of haddock the transition from the neck into the trunk is more gradual and there is usually no bulge present, or only a slight one. The formation of the bulge in the parasite of whiting might be caused by, and is usually associated with, the sharp flexure in that area. The angle between the neck and the anterior part of the trunk is very acute in *Lernaeocera* of whiting and far less acute in that of the haddock. To check this difference quantitatively the angles of flexure were measured in over 40 parasites of haddock and over 50 of whiting. The measurements can be summarized as follows:

	Range	Mean
Haddock	58–142°	93°
Whiting	0–120°	27°

MORPHOLOGY OF THE ADULT MALE

The discovery of a separate intermediate host for the *Lernaeocera* of haddock led to the investigation of possible morphological differences between the parasites from lemon sole and flounder. The mature males and females are found among the developmental stages of *Lernaeocera* on the intermediate host, and the former were chosen for the purpose of comparison. It was felt that the earlier stages might be less reliable as far as diagnostic features were concerned. The mature females from the intermediate host differ greatly in the degree of elongation of the genital segment, and for that reason are not easy to use in comparison of the bodily proportions.

No difference in the structure of the cephalothoracic appendages or thoracic limbs was found. The flounder *Lernaeocera* were more heavily pigmented, with the pigment extending into the thoracic legs. The legs of the lemon sole *Lernaeocera* were free from pigment and there was much less pigment in the thorax and abdomen.

Measurements were made on 247 mature males, 97 from lemon soles and 150 from flounders. The males from individual flounders were kept in separate groups (50 from one fish and 10 each from the remaining fishes). No such separation was possible for the lemon sole parasites, since there were never sufficient numbers of mature males on one fish to obtain representative mean values.

Two separate series of measurements were made. The first series contained measurements of 47 males from lemon soles and 100 from flounders. For this series the following measurements were taken on each individual: (i) total length, (ii) cephalothorax length, (iii) total length minus cephalothorax, (iv) cephalothorax width, (v) abdomen width.

The second set comprised 50 individuals from lemon soles and 50 from flounders. For this series the following measurements were made on each individual: (i) total length, (ii) cephalothorax length, (iii) free thorax length, (iv) abdomen length, (v) cephalothorax width, (vi) abdomen width.

The mean values and the standard errors for these measurements are given in Tables 4 and 5. These tables show that the means for all the characters are higher for the flounder parasites than for those of the lemon soles in both series. In most, though not all, cases the differences between means for the same character differ significantly for the two types of male. The first quantitative observations had suggested the existence of marked differences between the length and width of cephalothorax and between the length of the latter and the remainder of the body in the two types. Ratios were calculated for both pairs in each series and the values, together with the standard errors, are given in Tables 6 and 7. For lemon sole parasites the mean values of both ratios are significantly higher than for any of the flounder parasites.

The major object in taking these measurements was to ascertain whether the parasites from the two host species were distinct and then to ascertain whether such a distinction, if it existed, was confined to one or shown by several characters. In particular, it was important to find out whether the parasites from different flounders resembled one another more closely than they did parasites from lemon soles.

In Tables 8 and 9 are shown the maximum differences between the mean values of the various characters for lemon sole and flounder parasites. The differences for the various measurements have been standardized to allow direct comparisons between them.

These tables show that the separation between the two parasite populations is greatest in the case of abdomen width. Cephalothorax width is also a good discriminating character in series 1, although not so good in series 2 because of the presence in it of one flounder group providing an exceptionally low mean value for this character (0·410 mm). Good discrimination is also provided by the total length minus cephalothorax in series 1, and Table 9 shows that this difference is confined to the length of the free thorax. On the other hand, cephalothorax length does not discriminate well between the two types of parasites. It follows that good discrimination will be provided by any ratio involving abdomen width.

A useful and efficient measure of the differences between the groups of observations has been proposed by Mahalanobis (1930) and called by him the generalized distance. Not only does this quantity take into account any desired number of characters at the same time, but it also gives appropriate weight to different biological characters and takes into account the possible correlations between them (which is not done by a ratio).

If any one character is considered, the mean values for different groups may be arranged as points along a line, the distance of a point from the origin being equal to the mean for that group. The distance between two groups is then the distance between the points representing their mean values. To allow the comparison of distances for different characters all scales of measurement are equalized by dividing measurements by their standard deviations. When

TABLE 4. ADULT MALES, SERIES 1. MEAN VALUES AND STANDARD ERRORS (MM) FOR THE DIFFERENT CHARACTERS

Character		Lemon sole (47)	Flounder (50)	Flounder (10)	Flounder (10)	Flounder (10)	Flounder (10)	Flounder (10)
Total length	Mean	1·298	1·478	1·484	1·490	1·488	1·446	1·430
	S.E.	±0·0060	±0·0058			±0·0130		
Abdomen width	Mean	0·172	0·208	0·212	0·208	0·210	0·204	0·208
	S.E.	±0·0011	±0·0010			±0·0023		
Cephalothorax width	Mean	0·376	0·440	0·442	0·423	0·426	0·426	0·441
	S.E.	±0·0025	±0·0024			±0·0054		
Total length minus cephalothorax	Mean	0·657	0·801	0·812	0·820	0·819	0·800	0·788
	S.E.	±0·0041	±0·0040			±0·0089		
Cephalothorax length	Mean	0·622	0·674	0·672	0·670	0·669	0·646	0·642
	S.E.	±0·0040	±0·0038			±0·0086		

two or more characters are used this method of representation may be extended to two or more dimensions.

For the present data there are seven groups in series 1 and six in series 2. In order to give a measure of distance between lemon sole groups the data in

TABLE 5. ADULT MALES, SERIES 2. MEAN VALUES AND STANDARD ERRORS (MM) FOR DIFFERENT CHARACTERS

Character		Lemon sole (50)	Flounder (10)	Flounder (10)	Flounder (10)	Flounder (10)	Flounder (10)
Total length	Mean	1·302	1·503	1·494	1·468	1·440	1·390
	S.E.	±0·0055	±0·0122				
Abdomen width	Mean	0·168	0·220	0·210	0·210	0·204	0·198
	S.E.	±0·0011	±0·0024				
Cephalothorax width	Mean	0·372	0·440	0·438	0·432	0·435	0·410
	S.E.	±0·0018	±0·0041				
Free thorax length	Mean	0·322	0·357	0·376	0·375	0·368	0·366
	S.E.	±0·0024	±0·0054				
Abdomen length	Mean	0·352	0·458	0·456	0·444	0·434	0·396
	S.E.	±0·0027	±0·0059				
Cephalothorax length	Mean	0·627	0·688	0·662	0·650	0·639	0·628
	S.E.	±0·0040	±0·0090				

TABLE 6. SERIES 1. MEAN RATIOS AND STANDARD ERRORS

Host	Sample size	$\frac{\text{Cephalothorax length}}{\text{Cephalothorax width}}$	$\frac{\text{Cephalothorax length}}{\text{Total length minus cephalothorax}}$
Lemon sole	47	1·65±0·011	0·92±0·007
Flounder (1)	50	1·54±0·011	0·84±0·006
Flounder (2)	10	1·52±0·022	0·83±0·020
Flounder (3)	10	1·59±0·040	0·82±0·016
Flounder (4)	10	1·57±0·011	0·82±0·005
Flounder (5)	10	1·52±0·020	0·81±0·010
Flounder (6)	10	1·46±0·018	0·82±0·011

TABLE 7. SERIES 2. MEAN RATIOS AND STANDARD ERRORS

Host	Sample size	$\frac{\text{Cephalothorax length}}{\text{Cephalothorax width}}$	$\frac{\text{Cephalothorax length}}{\text{Total length minus cephalothorax}}$
Lemon sole	50	1·68±0·012	0·93±0·007
Flounder (1)	10	1·57±0·028	0·85±0·023
Flounder (2)	10	1·51±0·023	0·80±0·012
Flounder (3)	10	1·50±0·014	0·79±0·007
Flounder (4)	10	1·47±0·013	0·80±0·011
Flounder (5)	10	1·54±0·029	0·82±0·011

TABLE 8. SERIES 1. DIFFERENCES BETWEEN STANDARDIZED MEAN VALUES FOR DIFFERENT CHARACTERS

Character	Maximum difference between flounder parasites	Minimum difference between flounder and lemon sole parasites
Abdomen width	1·08	4·33
Cephalothorax width	1·12	2·76
Total length minus cephalothorax	1·15	4·03
Cephalothorax length	1·18	0·73

TABLE 9. SERIES 2. DIFFERENCES BETWEEN STANDARDIZED MEAN VALUES FOR DIFFERENT CHARACTERS

Character	Maximum difference between flounder parasites	Minimum difference between flounder and lemon sole parasites
Abdomen width	2·90	3·94
Cephalothorax width	2·31	2·92
Free thorax length	1·11	2·05
Abdomen length	3·30	2·34
Cephalothorax length	2·12	0·03

TABLE 10. DISTANCES BETWEEN GROUPS BASED ON CEPHALOTHORAX LENGTH, LENGTH OF REST OF BODY AND CEPHALOTHORAX WIDTH

(In arbitrary units)

(LS)1		(LS)2		F11		F12		F13		F14		F15	
(LS)2	0·7	(LS)1	0·7	(LS)1	5·3	(LS)1	5·8	(LS)1	5·0	(LS)1	5·2	(LS)1	4·5
F11	5·3	F11	5·9	(LS)2	5·9	(LS)2	6·2	(LS)2	5·5	(LS)2	5·7	(LS)2	5·1
F12	5·8	F12	6·2	F12	0·4	F11	0·4	F11	1·2	F11	1·1	F11	1·2
F13	5·0	F13	5·5	F13	1·2	F13	1·3	F12	1·3	F12	1·1	F12	1·4
F14	5·2	F14	5·7	F14	1·1	F14	1·1	F14	0·3	F13	0·3	F13	1·2
F15	4·5	F15	5·1	F15	1·2	F15	1·4	F15	1·2	F15	1·2	F14	1·2
F16	5·4	F16	6·0	F16	1·2	F16	1·1	F16	1·8	F16	1·7	F16	0·9
F21	6·6	F21	7·2	F21	1·8	F21	1·5	F21	1·7	F21	1·5	F21	2·5
F22	5·4	F22	6·0	F22	0·5	F22	0·4	F22	1·1	F22	1·0	F22	1·0
F23	5·3	F23	5·9	F23	1·0	F23	1·0	F23	1·1	F23	0·9	F23	0·8
F24	4·8	F24	4·8	F24	1·3	F24	1·4	F24	1·4	F24	1·7	F24	0·8
F25	3·5	F25	3·5	F25	2·3	F25	2·6	F25	1·9	F25	2·0	F25	1·2

F16		F21		F22		F23		F24		F25	
(LS)1	5·4	(LS)1	6·6	(LS)1	5·4	(LS)1	5·3	(LS)1	4·8	(LS)1	3·5
(LS)2	6·0	(LS)2	7·2	(LS)2	6·0	(LS)2	5·9	(LS)2	5·4	(LS)2	4·2
F11	1·2	F11	1·8	F11	0·5	F11	1·0	F11	1·3	F11	2·3
F12	1·1	F12	1·5	F12	0·4	F12	1·0	F12	1·4	F12	2·6
F13	1·8	F13	1·7	F13	1·1	F13	1·1	F13	1·8	F13	1·9
F14	1·7	F14	1·5	F14	1·0	F14	0·9	F14	1·7	F14	2·0
F15	0·9	F15	2·5	F15	1·0	F15	0·8	F15	0·8	F15	1·2
F21	2·4	F16	2·4	F16	1·8	F16	0·8	F16	0·6	F16	2·1
F22	0·8	F22	1·7	F21	1·7	F21	1·9	F21	2·8	F21	3·5
F23	0·8	F23	1·9	F23	0·6	F22	0·6	F22	1·1	F22	2·2
F24	0·6	F24	2·8	F24	1·1	F24	1·0	F23	1·0	F23	1·8
F25	2·1	F25	3·5	F25	2·2	F25	1·8	F25	1·8	F24	1·8

LS, lemon sole; F, flounder; 12, denotes the second flounder of the first series, etc.

series 1 and 2 were combined in respect of cephalothorax length, cephalothorax width and the length of the rest of the body and the generalized distances (shown in Table 10) were calculated.

Clearly the points for the parasites of the lemon soles lie very close together and relatively quite far away from the nearest point of the flounder parasites. The shortest distance between the lemon sole parasites and those of the flounder is 3·5, equal to the distance between the two flounder parasite populations farthest apart. The average distance between flounder groups is 1·4.

Having shown that it is quite reasonable to consider the parasites of the flounders as being distinct from those of the lemon sole, it is of interest to find the character or combination of characters which most clearly brings out the difference between the two groups of parasites. To do this, the data from series 2 only were used and the measurements from the parasites of individual flounders combined into a single set. Analysis showed that all characters are useful for the purpose of discrimination, but the additional information in this respect given by cephalothorax length was relatively small. Using all characters, the combination which most clearly brings out the differences between the two groups was found to be

$$(\text{A.W.}) + 0{\cdot}400\,(\text{C.W.}) + 0{\cdot}376\,(\text{F.T.L.}) + 0{\cdot}177\,(\text{A.L.}) - 0{\cdot}145\,(\text{C.L.}),$$

where A.L. is abdomen length, A.W. abdomen width, F.T.L. free thorax length, C.L. cephalothorax length and C.W. cephalothorax width.

This combination is known as a discriminant function (Fisher, 1936). The mean value of this quantity for the lemon sole parasites of series 2 is 27·358 and for the flounder parasites is 33·468. The difference 6·110 has a standard error of ±0·221 and is therefore highly significant. In fact, using this function of the characters to classify an individual parasite of unknown origin, the probability of misclassification is about three in a thousand.

MODE OF ATTACHMENT

The specific name of *L. branchialis* refers to the mode of attachment of this parasite. It is described by Linnaeus (1767) as being located 'ad branchias'. Stekhoven (1936) doubts the fitness of the name and regards the species as a heart rather than a gill parasite. His histological examinations have revealed that the bulbus arteriosus is the main region of penetration by this parasite. In his later work (Stekhoven & Punt, 1937), however, he modifies his opinion and states that the parasite is very often found in other localities. This applies, according to him, primarily to the older hosts. The distance from the point of attachment to the bulbus becomes too great and the parasite is no longer capable of reaching it.

The following observations were made during the present investigations:

(1) Small whiting had the parasite implanted in the bulbus in the majority

of cases (over 75 %). One parasite penetrated the ventricle, the walls of which were pushed out by the antlers.

(2) Out of 295 of the mature female parasites from haddock only 19 were attached to the bulbus arteriosus (6·4 %), while the remaining ones penetrated mainly the ventral aorta and some were attached to the branchial arteries.

(3) In Icelandic cod 8 out of 25 parasites were found in the bulbus arteriosus (33·3 %). Here also one parasite was embedded in the ventricle.

When the above facts are considered, two things become clear:

(*a*) The percentage of parasites in the bulbus is far larger both for whiting and cod than for haddock.

(*b*) This difference is not due to environmental conditions. Both whiting and cod are infested at an early age, while haddock continues to be infested throughout its life.

Parasites which infest a larger host might be unable to penetrate the bulbus due to the distances involved. But it must be remembered that at least a half of the infested haddock become infested during the first two years of their lives. Even some of the old haddock bear mature parasites, with the necks almost as long as the rest of their bodies and fully capable of reaching into the bulbus area. Sometimes a parasite penetrates from one subopercular cavity into the other, so that its neck is twice the length necessary to reach the bulbus.

Reference to Fig. 6 shows that there is no great difference in the spatial relations between the main area of attachment of *Lernaeocera* and the cardiac region of whiting and haddock. It would appear that in whiting the parasite may even have to reach farther back to enter the bulbus than in the haddock; and, indeed, *Lernaeocera* infesting whiting are very often found in the bulbus even when it does not represent the most accessible spot. Fig. 5 shows that in whiting the neck of *Lernaeocera* is often bent at a right angle to the main axis of the body. Being attached at the same level as the heart it has to make a sharp bend in order to reach it. This phenomenon is almost suggestive of a tropism and, in fact, the term 'arterotropism' has been coined for it by Capart (1948).

The haddock *Lernaeocera*, generally with the same possibilities of reaching the heart, is usually implanted more anteriorly in the ventral aorta, although this frequently means that the neck has to travel a longer way. This results in a striking difference between the two parasites. The whiting parasite has a short neck bent at a right angle to the longitudinal axis of the body, and that of the haddock has a relatively longer neck which deviates only slightly from that axis.

THE SEPARATION OF THE SPECIES

In the preceding sections of this paper several definite and consistent differences between *Lernaeocera* infesting whiting and cod and *Lernaeocera* found on haddock were discussed. The differences were of such character and extent that they could only be explained by the existence of specific distinctions between these two parasites.

We have seen that the geographic distribution of the incidence of *Lernaeocera* in haddock differs greatly from that of cod and whiting. First, haddock is infested throughout the northern North Sea, while the other two hosts are are free from infestation in the open sea. Secondly, no infested haddock is found outside the European continental shelf, while cod of, for example, Faröe and Iceland are infested to the same, or larger, extent than in the North Sea. One can visualize the spread of *Lernaeocera* from the North Sea northwards. Infested cod carry over the mature parasites to Faröe, the eggs deposited there hatch and, in the presence of a suitable intermediate host (both lemon sole and flounder are present in that area), the parasite becomes established. Haddock, presumably owing to its inability to negotiate the deep water barriers, does not spread its own *Lernaeocera*. Only the specific distinctness of the parasite of whiting and cod can explain the absence of infestation in haddock outside the European continental shelf.

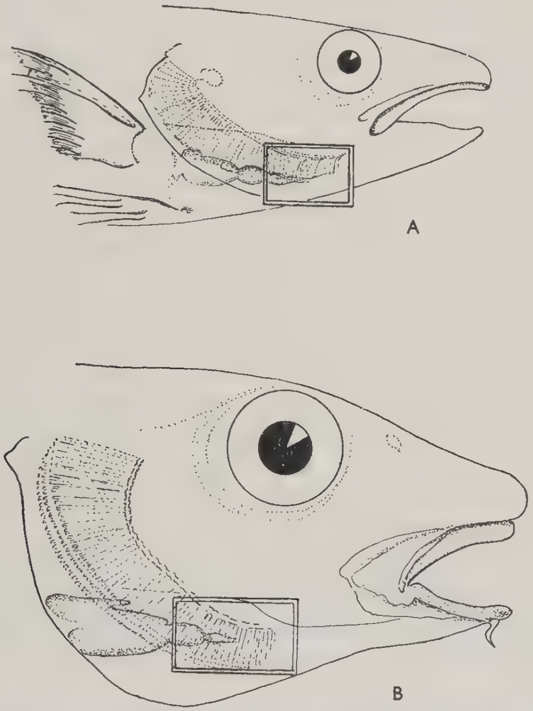

Fig. 6. Heads of (A) whiting and (B) haddock, showing the main attachment area of *Lernaeocera* and its relation to the position of the heart.

The three most important differences in the morphology of the two types of *Lernaeocera* were (i) the angles of the genital flexure, (ii) the relation between the ratios of thickness of the neck to that of the trunk, and (iii) the structural differences of the neck and trunk junction area.

Ginsburg (1938), proposing his definition of the arithmetical concept of species, subspecies and race, gives certain values for 'intergradation' of a particular character under comparison. These values are: 10% or less for specific difference, 15–25% for a subspecific one and 30–40% for a racial one. Comparison of the values for the angles of genital flexure (Fig. 3) shows them to intergrade to the extent of 18·8%. The values for the thickness ratios intergrade to 10%, while those for the neck flexure angles to 4·8% only. The first of them is, therefore, in the subspecific category, the remaining two in specific. The third of them is the best discriminant. In fact, the presence of the bulge in whiting *Lernaeocera* makes the difference even more pronounced than it appears from the angle measurements. Of the sample of 58 parasites of whiting 13 had necks which, in the junction area, were completely parallel to the anterior part of the trunks. In most of the specimens from whiting the neck bends again more anteriorly (Fig. 5), the angle of the bend being roughly inversely proportional to that of the neck flexure. In most of the parasites whose neck flexure exceeds 30°, the neck bends back on itself, its distal and proximal parts running almost parallel to each other.

Lernaeocera of cod and whiting, but not of haddock, is embedded predominantly in the bulbus arteriosus. To all appearances this difference is independent of environmental circumstances. It is interesting to speculate to what extent the structure of the neck region of the parasite of whiting is related to its penetration of the region of the bulbus. The characteristic flexure may result from the parasite 'striving' to reach the heart of the host; alternatively, it may penetrate the heart because of the flexure of the neck.

The mature males from their respective intermediate hosts differed not only in absolute size but in the proportions of the body and the extent of pigmentation.

Summing up all these points of difference, the author became convinced that the two parasites are specifically distinct. The existence of some distinguishing features between the two types of *Lernaeocera* has, however, been observed as early as the eighteenth century. O. Fabricius (1780, p. 336) and O. F. Müller (1789, p. 65) both regarded the parasite of the haddock as 'varietas minor' of *Lernaea gadina* (= *Lernaeocera branchialis*), but it appears that size was the only difference prompting these two authors to regard this parasite as distinct from those of '*Gadi barbati*'. The figure which O. F. Müller gives of his *L. gadina* (pl. CXVIII, fig. 4) is evidently that of the cod and whiting *Lernaeocera*.

The discovery that the name *L. branchialis* is at present used to cover two distinct, if very similar, species brings with it the problem of which of the two species should retain the old name and which should be given a new one.

The solution of this problem depends on the host species from which *L. branchialis* was first described. Linnaeus (1767), who first named the animal in the 12th edition of *Systema Naturae* (p. 1092), describes it as parasitizing: 'Gadis, ad branchias.' Possibly Linnaeus never actually saw the animal and details of it must be sought in Strøm (1762), to whom Linnaeus refers. Strøm is rather vague about the hosts on which the parasite occurs, stating that it is found 'i Fiskenes Tokn' (on gills of fishes). He adds later, however, 'besonderlig i Torskenes om Foraaret' (especially on cod in the spring). In view of this statement, which is the oldest on record concerning the host of *L. branchialis*, it seems proper to retain the old name for the parasite of whiting and cod and rename the parasite of haddock, which the author proposes to name *L. obtusa* n.sp. The name is suggestive of the characteristic wide angle of genital flexure.

Lernaeocera obtusa sp.nov.

Lernaea gadina (in part) Fabricius, 1780. (The reference to 'varietas minor', judging from its context, does not constitute a proposal of 'minor' as a name for *Lernaeocera* found on haddock.)

Lernaea gadina (in part) O. F. Müller, 1789. (The reference to 'varietas minor' as above.)

Lernaeocera branchialis (in part) Blainville, 1822. (A figure of *L. obtusa* is given, but Linnaeus 1767, following Strøm 1762, by not mentioning the haddock has implicitly restricted *L. branchialis* to the parasite of the cod.)

Lernaea branchialis (in part) T. & A. Scott, 1913.

Lernaeocera branchialis (in part) Oorde de Lint & Stekhoven, 1936.

Holotype: mature female deposited in British Museum (B.M. no. 1957.2.21.1). *Paratypes*: six mature males and females deposited in British Museum (N.H.) B.M. no. 1957.2.21.2. Six mature males and females deposited in Royal Scottish Museum, Edinburgh, No. Royal Scottish Museum 1957.24. *Type locality*: Northern North Sea. Collected by Z. Kabata.

Description. Female: size within the range of that of *L. branchialis*. Shape generally similar to that of *L. branchialis*, with the following exceptions: (1) The flexure of the neck and the anterior part of the trunk forms roughly a right angle (average of 58 specimens was 93°, with the range of 58–142°), while that of *L. branchialis* forms an acute angle or no angle at all, both parts running parallel to each other. (2) The neck is considerably thicker in relation to the diameter of the trunk than in *L. branchialis*. (3) The flexure of the genital segment forms usually an obtuse angle (average of 65 specimens was 99°), while that of *L. branchialis* forms an acute one.

Male: generally similar to the male of *L. branchialis*, but mean size 1·30 mm as compared with 1·46 of the latter. Cephalothorax longer in relation to the body length than in *L. branchialis*. Cephalothorax and abdomen narrower than in the latter species.

Intermediate host: lemon sole, *Microstomus kitt* (Walbaum); habitat: gills. Final host (female only): haddock, *Gadus aeglefinus* L.; habitat: branchial chamber or gills.

The author wishes to express his gratitude to the following members of the staff of the Marine Laboratory, Aberdeen: Mr J. A. Pope, who is responsible for the statistical analysis and largely for the form of the section on the morphology of the male, Dr B. B. Rae and Dr J. H. Fraser for their interest, encouragement, useful criticism and corrections of the manuscript.

Thanks are also due to Dr P. A. Orkin of the University of Aberdeen for reading the manuscript and for help in tracing literature, to Mr C. Muir, who drew Fig. 5, and to Dr D. W. Tucker of the British Museum for access to Fabricius's *Fauna Groenlandica*.

SUMMARY

The fishes harbouring parasites of the genus *Lernaeocera* can be divided, according to the presence or absence of contact with the coastal waters, into 'inshore' and 'offshore' groups. According to literature, only *L. branchialis* occurs commonly on hosts in both groups. The difference between this and the remaining species of the genus is explained by the existence of two different species covered by the name *L. branchialis*. One of these species is parasitic on cod and whiting, the other on haddock. The two species have also different intermediate hosts (flounder and lemon sole respectively) and differ in morphology of the adult male and female and in the mode of attachment to the final host.

Lernaeocera of whiting and cod is distributed mainly in the coastal area, while that from haddock extends over the whole of the North Sea. The former, but not the latter, is present outside the European continental shelf. All these differences lead to the conclusion that the two types represent two different species. The old name is retained for the parasite of cod and whiting. The parasite of haddock is the new species, to which the name *Lernaeocera obtusa* is given.

REFERENCES

Basset-Smith, P. W., 1896. Notes on the parasitic copepoda of fish obtained at Plymouth, with descriptions of new species. *Ann. Mag. nat. Hist.*, Vol. 18, pp. 8–16.

Blainville, H. M. D. de, 1822. Mémoire sur les Lernées (*Lernaea*, Linn.). *J. Phys. Chim. Hist. nat.*, T. 95, pp. 372–80 and 437–47.

Capart, A., 1948. Le *Lernaeocera branchialis* (Linné 1767). *Cellule*, Vol. 52, pp. 159–212.

Dollfus, R. P., 1953. Parasites Animaux de la Morue Atlanto-Arctique. *Encycl. biol.*, Vol. 43, pp. 350–63.

Fabricius, O., 1780. *Fauna Groenlandica*. 452 pp. Hafniae et Lipsiae.

FISHER, R. A., 1936. The use of multiple measurements in taxonomic problems. *Ann. Eugen., Lond.*, Vol. 8, pp. 179–88.

GINSBURG, J., 1938. Arithmetical definition of the species, subspecies and race concept with a proposal for modified nomenclature. *Zoologica, N.Y.*, Vol. 23 (13), p. 253.

GOUILLART, M., 1937. Recherches sur les Copépodes parasites. *Trav. Sta. zool. Wimereux*, T. 12, pp. 309–457.

GÜNTHER, A., 1862. *Catalogue of the Acanthopterygii Pharyngognathi and Anacanthini in the collection of the British Museum.* Vol. 4, 534 pp. London: British Museum.

KRØYER, H., 1863. Bidrag til Kundsgab om Snyltekrebsene. *Naturh. Tidsskr.*, Bd. 2, pp. 74–426.

LINNAEUS, C., 1767. *Systema Naturae*, 12th Ed.

MAHALANOBIS, P. C., 1930. On tests and measures of group divergences. *J. Asiat. Soc. Beng.*, Vol. 26, pp. 541–88.

MÜLLER, O. F., 1789. *Zoologia Danica*, Vol. 3.

OORDE DE LINT, G. M. VAN & SCHUURMANS STEKHOVEN, J. H., 1936. Copepoda parasitica. *Tierw. N.-v. Ostsee*, Teil 10c, pp. 73–198 (Lief. 31).

QUIDOR, A., 1912. Copepodes Parasites. *Deuxième Expédition Antarctique Française* (1908–1910), Paris, pp. 197–215.

RAE, B. B., 1939. Factors in the growth of lemon soles. *Sci. Invest. Fish. Scot.*, 1939, No. 3, 18 pp.

SCOTT, A., 1901. *Lepeophtheirus* and *Lernaea. L.M.B.C. Memoir*, No. 6.

SCOTT, T., 1900. Notes on some crustacean parasites of fishes. *Rep. Fish. Bd Scot.*, No. 18, pp. 144–88.

SCOTT, T. & SCOTT, A., 1913. *British Parasitic Copepoda.* London: Ray Society.

SHULMAN, S. S. & SHULMAN-ALBOVA, R. E., 1953. *Parasites of Fishes of the White Sea.* 192 pp. Acad. Sci. U.R.S.S. Moscow. [In Russian.]

SPROSTON, N. G., 1941. The developmental stages of *Lernaeocera branchialis. J. mar. biol. Ass. U.K.*, Vol. 25, pp. 441–66.

SPROSTON, N. G. & HARTLEY, P. H. T., 1941. The ecology of some parasitic copepods of gadoids and other fishes. *J. mar. biol. Ass. U.K.*, Vol. 25, pp. 361–92.

STEKHOVEN, J. H. SCHUURMANS, 1935. Copepoda parasitica from the Belgian coast. *Bull. Mus. Hist. nat. Belg.*, T. 11, No. 7, 13 pp.

—— 1936. Beobachtungen zur Morphologie und Physiologie der *Lernaeocera branchialis* L. und *Lernaeocera lusci* Basset-Smith. *Z. Parasitenk.*, Bd. 8, pp. 659–96.

STEKHOVEN, J. H. SCHUURMANS & PUNT, A., 1937. Weitere Beiträge zur Morphologie und Physiologie der *Lernaeocera branchialis* L. *Z. Parasitenk.*, Bd. 9, pp. 648.

STRØM, H., 1762. *Physisk og Oeconomisk Beskrivelse over Fogderiet Søndmør, beliggende i Bergens Stift i Norge.* Første Part. Copenhagen.

TEMPLEMAN, W., 1953. Knowledge of division of stocks of cod, haddock, redfish and American plaice in subareas 3 and 2 of the Northwest Atlantic Convention area. *Ann. Proc. int. Comm. N.W. Atlant. Fish.*, Vol. 3, pp. 63–5.

THOMSON, G. N., 1889. Parasitic Copepoda of New Zealand. *Trans. Proc. N.Z. Inst.*, Vol. 22, pp. 353–75.

WILSON, C. B., 1917. North American parasitic copepods belonging to the Lernaeidae with a revision of the entire family. *Proc. U.S. nat. Mus.*, Vol. 53, pp. 1–150.

Note added in proof

Since this paper was written, another interesting fact has been brought to light. Investigations in Faröe waters revealed *Lernaeocera* on the gills of the lemon sole. No other flatfish species has yet been found to carry this parasite in that area. The biometric measurements of the adult males found on the lemon sole in Faröe wateres proved them to resemble those of the lemon soles of the North Sea.

A suggestion has been put forward in this paper (p. 573) as to the possible way in which *L. branchialis* spread northwards from the North Sea. It is only larger and older cod which can be regarded as possible carriers of the parasite across the deep water barrier which confines the host of *L. obtusa* within the limits of the continental shelf. Such cod, crossing to Faröes, are unlikely to enter the littoral zone and come in contact with the flounder. The parasite will therefore deposit its eggs in deeper waters and its copepodids, to survive, must become adapted to a host available in those waters. The lemon sole, as the most common local flatfish species, became an intermediate host. The fact that, in spite of the infestation of the lemon sole, no parasite is found on the haddock, strongly suggests that the larvae on that flatfish belong to *L. branchialis.*

The biometric similarity between the parasites found on the lemon soles in Faröes and the North Sea suggests that the bodily proportions of the male are subject to environmental influences and cannot be used as specific discriminant. The conclusions of the section of this paper dealing with the morphology of the male appear therefore to be incorrect.

While not affecting the main argument of this paper, the discovery focuses attention on the interesting and little-known problems of ecology of marine parasites.

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NOTES ON PRACTICAL METHODS FOR THE STUDY OF MARINE DIATOMS

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In the study of marine diatoms examination of fresh material (or of wet mounts in some suitable preservative such as formalin–sea water) is essential, in addition to 'permanent' mounts of 'cleaned' frustules, because the violent cleaning process destroys many of the more delicate forms (e.g. the genus *Guinardia*). Conversely, it is not possible to see the fine details of skeletal structure, upon which the taxonomy of so many diatoms is based, while the organic contents of the cells are still present.

Scatter-mounts are preferred to arranged slides because the examination of large numbers of individuals is essential if the wide range of variation within species of this group is to be justly appreciated (cf. Hendey, 1938, 1951). Further, they help one to appreciate the relative abundance of species within the sample, provided that wet mounts have previously been examined.

The 'cleaning'—removal of the organic contents of the frustules—may be carried out by direct incineration in a platinum crucible, but some form of wet combustion is usually found better and more convenient. The successive treatments with strong mineral acids and powerful bleaching agents that this involves can be carried out satisfactorily as described by Hendey (1938, 1951) if there is ample material available. Frequently, however, in marine biological work, it is necessary to use very small quantities; for example, the gut contents of a few small invertebrates, or the epiphytes from the shell of a single large barnacle.

It is possible to apply the same schedule of successive washings and treatment with acids that Hendey recommends, to smaller amounts of material, if one's facilities include the use of a small centrifuge and a fume cupboard. The reagents can safely be added (in the very small quantities needed) to material in a 12 ml. centrifuge tube directly with a pipette provided the mouth of the tube is kept pointing away from the face of the operator, towards the far side of the fume cupboard. Much time is saved by concentrating the material centrifugally between each stage instead of waiting for it to settle before decanting off the unwanted fluids. The elimination of sulphuric acid is especially facilitated by the small quantity (*ca.* 4 ml.) of reagent needed. One can dilute it by cautiously pipetting water into it in the tube, allowing it to 'kick' slightly and boil momentarily until the tube is gradually filled. This eliminates most of the possibility of trouble from spurting acid when, after bringing the material down again with the centrifuge, the acid is finally poured off. With the speeding up of the process that use of the centrifuge permits, extra stages, such as treatment with a fat-solvent or extra washing with distilled water can easily be interpolated at appropriate points in the schedule.

Centrifuge tubes of Pyrex glass, scrupulously cleaned, should be used; and it is important to make sure that their ends are adequately cushioned against direct contact with the bottoms of the buckets. It is essential to use one newly drawn or *very* thoroughly cleaned pipette for all stages in the manipulation of each separate sample.

By these means good cleaned samples of marine diatom frustules ready for mounting have been obtained from material as diverse as plankton samples, epiphytes, scrapings from the skin-film of whales and diatomaceous bottom muds; working with very small quantities of material when necessary.

The material, once cleaned and washed, can conveniently be stored in very small vials, labelled, corked and waxed, in either distilled water or alcohol. Alcohol is preferred because it prevents the growths that may take place if the material is stored in water for a long time.

Before mounting dehydration is necessary and this can be completed by direct heating and use of a desiccator filled with fresh silica gel. Several cleaned cover-glasses are placed on black paper in a small petri dish, one large drop of the material, agitated until the diatom frustules cause a faint milkiness of the fluid, is pipetted on to each, the dish covered and heated at just above 100° C for a time, then removed to the desiccator. The slips are now ready for mounting by placing a small drop of the chosen medium near the centre of each, inverting it on to a cleaned slide and proceeding with the appropriate after-treatment (hardening, usually by gentle heat, and perhaps ringing).

There remains the choice of a suitable mountant, and here we are faced with the old problem resulting from the fact that diatom-silex has a refractive index quoted as around 1·40–1·42, so near to that of the best general mountants (e.g. Euparal 1·46 and Canada balsam 1·52) that the index of visibility of frustules mounted in them is too low. A mountant of much higher refractive index is needed.

Much patient experiment with mixtures of sulphur, phosphorus and alkaloids with other substances, usually resinous, and with fluids such as monobromonaphthalene, has been carried out in the past by such workers as Morris (1885) and Bellido (1897, 1927). The latter's work was translated into English (1927) and is extensively quoted by Gray (1953). Bellido's methods resulted in perhaps the finest diatom mounts ever made, rivalling the famous arranged slides of Müller. They consisted essentially of mounting in monobromonaphthalene sealed in hardened shellac cells. The technique is laborious and time-consuming, however, demanding a manipulative skill that can only be acquired through months of practice. The shellac also needs special purification.

In the meantime 'Styrax', and 'Hyrax', a proprietary resin of high refractive index, became popular with several workers. The use of Styrax is described in the introductory chapters of Hustedt's monograph (1930). We have had some good results with these media; but Styrax is difficult to control in the drying-off stages, when bubbles are frequently drawn in under the cover-glass, and it is inclined to give rather a dark ground colour. Moreover, Hustedt (1952) has since found Hyrax inadequate for some forms.

Ghazzawi (1933) revived an old method that dates back to the time of Morris's experiments, a solid but thermoplastic medium of good keeping qualities, obtained by grinding antimony tribromide with the alkaloid piperine. We found that this demanded very careful control of heating, difficult to apply in practice, and that the post-war price of piperine was prohibitive.

Jelly (1930), working on sections of cellulose nitrate film for Kodak Ltd., got good results by melting Canada balsam with alphabromonaphthalene (15 g to 10 ml.) on the water-bath. He suggested that this mixture, which gave him a refractive index of $N_D = 1{\cdot}591$, might also be useful with diatomaceae and ice crystals. I have had moderate success with it (Hart, 1935), but found that some mounts set poorly and tended to develop globules unless the proportions were modified, using a smaller quantity of a bromonaphthalene in balsam already made up with xylene. Some of the mounts thus obtained have lasted well for over 20 years, but the gain in refractive index is too small to give the best results with delicate frustules.

Latterly, the introduction of synthetic resins has led to the introduction of various mountants mostly known only under proprietary names, at least two of which were evolved expressly for the use of diatomists, by authors who published directions for their small-scale synthesis. These are 'Naphrax' (Flemming, 1943) and 'Pleurax' (Hanna, 1949). Pleurax was used extensively by Dr Cupp in the work leading up to her monograph on the marine diatoms of the Pacific coast of North America (Cupp, 1943), which should be a strong recommendation. A sample of Naphrax obtained commercially was very dark-coloured, which detracted from its otherwise excellent optical qualities. We hope to repeat the syntheses described by Fleming and Hanna eventually, in order to test Naphrax and Pleurax against the media described below.

Another proprietary mountant tried has been 'Clearax'—water white with a refractive index stated to be 1·66 when set. It gave good results, but other media seemed better.

For a long time I had failed to find Hanna's description of the small-scale synthesis of his Pleurax, and it was this that led me to experiment on lines suggested partly by Jelly's work, and partly by that of Kirkpatrick & Lendrum (1939). The latter developed a most useful cheap substitute for balsam (DPX, also 'DePex') for bacteriological and histological mounting by dissolving distrene-80, one of the polystyrenes already available commercially, in xylol, with the addition of a small proportion of tritolyl phosphate as plasticizer.

This medium is especially good for preserving the colour of some delicate stains that tend to fade in balsam; its refractive index, however, is but little higher than that of ordinary Canada balsam in xylol.

It was found that the synthetic resin could be dissolved in *a*-bromonaphthalene, and that the resultant syrup was miscible with xylene. Further, it could also be dissolved in *a*-iodonaphthalene, introducing a darker colour but an even higher refractive index. Finally, a useful medium for diatom mounts was achieved by mixing the three solutions obtained by dissolving distrene-80 to saturation in xylene, *a*-bromonaphthalene and *a*-iodonaphthalene, with the aid of gentle heat, in the proportions: two parts xylene solution, two parts *a*-bromonaphthalene solution, one part *a*-iodonaphthalene solution.

The mounts should be dried off at temperatures not greatly exceeding 100° C for about 1 h, and baked again similarly on the following day. If they are heated too strongly or too long immediately after mounting, bubble trouble may result. The mixed medium seems stable in an ordinary balsam-bottle. It may be thickened slightly by evaporating off some of the xylene, or thinned by adding more xylene as necessary.

The mounts seem good optically and have lasted for several months without being ringed. Ringing with gold-size may increase their keeping properties. Two out of some fifty have shown development of minute globules, such as Morris complained of in fluid mounts made with monobromonaphthalene alone, and which he avoided by incorporating a minute proportion of salicilate of chinoline in the mountant. With these polystyrene mixtures, it is thought that the defect was due rather to inadequate dessication before mounting, than to the quality of the naphthalene derivatives, which are obtainable in a much more pure state than in Morris's day.

The heavy halogen naphthalene derivatives have also been tried again mixed with Canada balsam in high proportions, as suggested by Jelly. This gave quite useful mounts but several developed globules in time, and all showed much more background colour than those made from the synthetic resin mixture. Other promising experiments have been made using venetian turpentine (more soluble than Canada balsam in ethylalcohol) as the resinous base, with the same optically effective liquids. It was hoped that by this means material stored in alcohol might be mounted more readily, since complete dessication would not be such a strict necessity. Some good mounts resulted, but dark background colour was again found to be a considerable disadvantage.

Only time can show the keeping qualities of the successful mixture of distrene-80 with xylene and the heavy halogen derivatives of naphthalene. Our next step must be to complete the small-scale syntheses of 'Naphrax' and Pleurax as so generously described by Drs Fleming and Hanna and see how they compare with our mountant. At worst the distrene-80 mixture provides a first class examination medium easy to prepare and far superior to temporary fluid mounts, even though they may not prove fully 'permanent'.

Throughout the later experiments Prof. Gray's source reference book has been a great help. I should like to thank the Director of the Plymouth Laboratory of the M.B.A., where the work was begun years ago; and also Dr Cox, chemist on the N.I.O. staff, for advice and encouragement.

REFERENCES

BELLIDO, DON ERNESTO CABALLERO, 1897. In *An. Soc. esp. Hist. nat.* [Not seen, a subsequent Spanish ed. appeared 1926, and an English translation quoted below.]

—— 1927. The technique of mounting diatom and other type slides. [Communicated by Dr C. Tierney from trans. by Mr Frederick Adams.] *J. R. micr. Soc.*, Vol. 47, pp. 9–28.

CUPP, E. E., 1943. Marine plankton diatoms of the West Coast of North America. *Bull. Scripps Instn Oceanogr.*, Vol. 5, pp. 1–238.

FLEMING, WILLIAM D., 1943. A synthetic mounting medium of high refractive index. *J. R. micr. Soc.*, Vol. 63, pp. 34–7.

GHAZZAWI, F. M., 1933. The littoral diatoms of Liverpool and Port Erin shores. *J. mar. biol. Ass. U.K.*, Vol. 19, pp. 165–76.

GRAY, PETER, 1953. *The Microtomists' Formulary and Guide.* London: Constable and Co.

HANNA, G. D., 1949. A synthetic resin which has unusual properties. *J. R. micr. Soc.*, Vol. 69, pp. 25–8.

HART, T. J. 1935. On the diatoms of the skin-film of whales, and their possible bearing on problems of whale movements. '*Discovery*' *Rep.*, Vol. 10, pp. 247–82.

HENDEY, N. INGRAM, 1938. An efficient technique for cleaning diatoms. *J. R. micr. Soc.*, Vol. 58, pp. 49–52.

—— 1951. Littoral diatoms of Chichester Harbour with special reference to fouling. *J. R. micr. Soc.*, Vol. 71, pp. 1–86.

HUSTEDT, FR., 1930. Die Kieselalgen Deutschlands, Österreichs und der Schweiz, *Rabenhorst's Kryptogamenflora*, Bd. 7, Teil 1, Leipzig.

—— 1952. Diatomeen aus der lebensgemeinshaft des Buckelwals (*Megaptera nodosa* Bonn). *Arch. Hydrobiol.*, Bd. 46, pp. 286–98.

JELLY, EDWIN E., 1930. Mounting medium for film sections. *Nature, Lond.*, Vol. 125, p. 276.

KIRKPATRICK, J. & LENDRUM, A. C., 1939. A mounting medium for microscopical preparations, giving good preservation of colour. *J. Path. Bact.*, Vol. 49, p. 593.

MORRIS, WILLIAM, 1885. Notes on experiments in mounting the *Amphiplura pellucida* in media having a higher refractive index than Canada balsam. *J. roy. Soc., N.S.W.*, Vol. 19, pp. 121–33.

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THE OCCURRENCE OF *IDOTEA METALLICA* BOSC IN BRITISH WATERS

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(Text-figs. 1 and 2)

Idotea metallica Bosc occurs occasionally in plankton from waters off the west coast of Britain, but since it does not seem to be a British resident it was excluded from a recent review of the genus *Idotea* (Naylor, 1955*a*). Accounts of the distribution of *I. metallica* seldom distinguish between occasional records and resident breeding localities, and this note attempts to explain its occasional occurrence in Britain.

I am grateful to Dr J. H. Fraser, Dr K. M. Rae and Mr G. M. Spooner for the loan of material, and to Dr I. Gordon for facilities at the British Museum. Prof. E. W. Knight-Jones and Dr R. J. Menzies have kindly criticized the manuscript.

SPECIFIC CHARACTERS (Fig. 1)

Body oblong; length about three times the width, except in females which are relatively much broader. *Cephalon* about $1\frac{3}{4}$ times as broad as long; anterior border concave, posterior border less so; marked transverse sinuous furrow behind the eyes; eyes large. *Antennules* hardly extending beyond the third joint of the antennal peduncle; first and second joints expanded, others fairly robust. *Aesthetascs* in pairs, numbering up to 20 or more in males; fewer in females. *Antenna* robust, flagellum shorter than peduncle and about one-sixth the length of the body; flagellum segments numbering up to about 10 in males and 8 in females; terminal style blunt, one quarter to one-sixth the length of the subterminal segment. *Coxal plates* triangular, extending over the whole length of the segment in segments 2 or 3 and posterior ones, becoming only slightly wider posteriorly; 5–7 sharply produced laterally making an angle of less than 45°. *Legs* robust; second leg of adult males having pads of fine setae. *Abdomen* with straight sides; *telson* with apical border straight, with rounded corners (adults) or widely rounded corners (juveniles). *Appendix-masculinus* reaches just beyond the tip of the second pleopod in larger males.

Length: males recognizable from about 8 mm, ranging to about 30 mm; females from 9 to 19 mm.

Colour: uniformly greyish or brown.

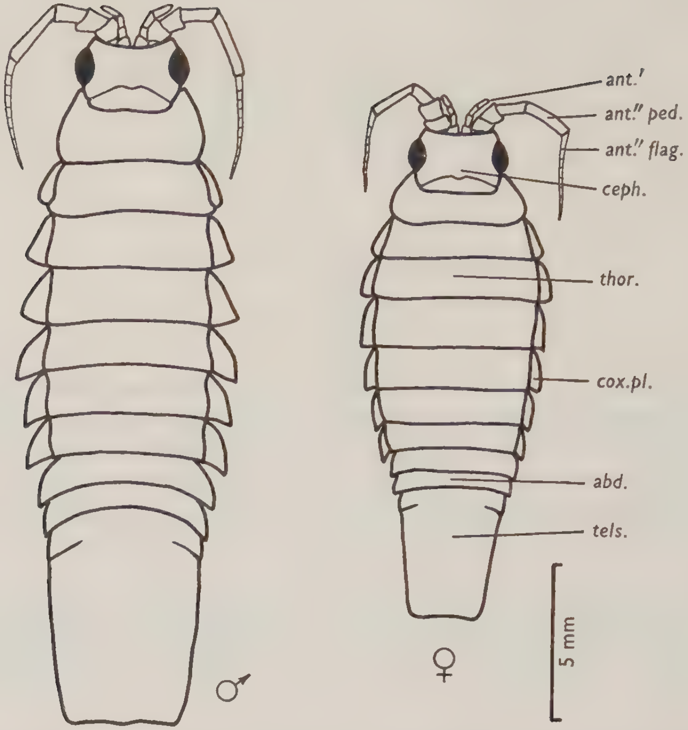
ant.'
ant." ped.
ant." flag.
ceph.
thor.
cox.pl.
abd.
tels.
5 mm
♂
♀

Fig. 1. Adult male and female *Idotea metallica*: *ant.'*, antennule; *ant."ped.*, antennal peduncle; *ant."flag.*, antennal flagellum; *ceph.*, cephalon; *thor.*, thorax; *cox.pl.*, coxal plate; *abd.*, abdomen; *tels.*, telson.

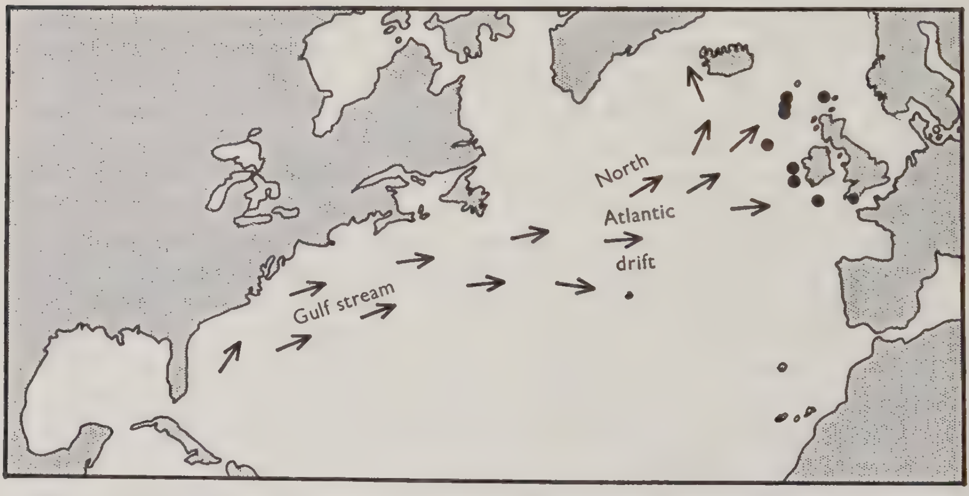
North
Atlantic
drift
Gulf stream

Fig. 2. The occurrence of *Idotea metallica* in British waters. (●, confirmed records of single or 'a few' specimens, from data in Table 1 and in Tattersall, 1906.)

GEOGRAPHICAL DISTRIBUTION

The distribution of *I. metallica* is described by Richardson (1905), Tattersall (1906, 1911), Stephenson (1915), Collinge (1917), Nierstrasz & Stekhoven (1930) and Cărăusu (1955). The species is known to breed in the Black Sea, Adriatic, Mediterranean and along the east coast of North America probably from Florida to Nova Scotia. In the north Atlantic, besides the occasional records from off the west coasts of Britain, there are rare records from off the coasts of Iceland and Greenland. There are also records from the west coast of North America, Patagonia, India and Australia. I have seen specimens from off the west coast of Britain (Table 1), off Plymouth (Marine Biological Association, 1957, p. 200), from the Atlantic, the east coast of North America, the Mediterranean, Montevideo and north-west Australia.

TABLE 1. BRITISH MATERIAL EXAMINED

Material	Location	Date	Source
2♀♀, 3♂♂	55° 49′ N., 16° 44′ W.	1875	British Museum ('Valorous' Expedition)
1 ♂	Near Labadie Bank	2. vii. 50	British Museum (Manihine Collection—as *I. pelagica*)
1 juv.	Bofin Is., C. Galway	1908	British Museum (Norman Collection)
1 juv.	60° 51′ N., 9° 06′ W.	15. vii. 49	Marine Laboratory, Torry, Aberdeen
1 ♂	61° 48′ N., 9° 06′ W.	11. ix. 50	Marine Laboratory, Torry, Aberdeen
1 ♂	59° 45′ N., 3° 00′ W.	7. ix. 54	Marine Laboratory, Torry, Aberdeen
1 ♀	59° 02′ N., 9° 08′ W.	9. vii. 55	Marine Laboratory, Torry, Aberdeen
1 juv.	2 miles E. of Eddystone	7. xi. 49	Marine Biological Laboratory, Plymouth (routine haul with 2 m stramin ring trawl)
1 ♂	49° 14′ N., 5° 11′ W.	2. v. 57	Plymouth Lab., vertical haul with coarse tow-net.

The species is variously recorded from amongst floating weed or timber, amongst floating colonies of *Lepas* (Tattersall, 1906, 1911), or even swimming freely at the surface (Menzies & Dow, 1957); and this surface living habit probably accounts for its wide distribution. Of the other species of *Idotea* in North Atlantic waters *I. baltica* is the only one which seems to live predominantly amongst drift weed (Naylor, 1955*b*), and this species is almost as widespread as *I. metallica* (Cărăusu, 1955). It seems likely that specimens of *I. metallica* reach British waters from the east coast of North America amongst floating debris carried by the North Atlantic Drift (Fig. 2), in rather the same manner as the grapsoid crab *Planes minutus* Leach (Bell, 1853) and several other organisms (Hardy, 1956) are thought to reach these waters. Some of Stebbing's *I. metallica* material in the British Museum is labelled 'Gulf

Stream'. British specimens belong to the form *typica* which is also found on the east coast of North America and in the western Mediterranean; a second form, *elongata*, is restricted to the Black Sea and eastern Mediterranean (Cărăusu, 1955). Though male and ovigerous female *I. metallica* have been occasionally recorded off Britain for the past hundred years or so, the species has not so far established itself.

REFERENCES

Bell, T., 1853. *A History of the British Stalk-eyed Crustacea*, 386 pp. London: Van Hoorst.

Cărăusu, A., 1955. Contributii la studiul isopodelor (Crustacea Malacostraca) Mării Negre (litoralul romînesc si regiunile învecinate). III. Familia Idoteidae. *Anal. Stiint. Univ. 'Al. I. Cuza' Din Iasi*, Sect. II, T. I, fasc. 1–2, pp. 137–216.

Collinge, W. E., 1917. A revision of the British Idoteidae. *Trans. roy. Soc. Edinb.*, Vol. 51, pp. 721–60.

Hardy, A. C., 1956. *The Open Sea*. 335 pp. London: Collins.

Marine Biological Association, 1957. *Plymouth Marine Fauna*, 3rd ed. Plymouth.

Menzies, R. J. & Dow, T. G., 1957. The pelagic isopod *Idotea metallica* in the Mediterranean. *Pubbl. Sta. zool., Napoli* (in the Press).

Naylor, E., 1955*a*. The comparative external morphology and revised taxonomy of the British species of *Idotea*. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 467–93.

—— 1955*b*. The ecological distribution of British species of *Idotea* (Isopoda). *J. Anim. Ecol.*, Vol. 24, No. 2, pp. 255–69.

Nierstrasz, H. F. & Stekhoven, J. H. Schuurmans jun., 1930. Isopoda genuina. *Tierwelt N.- u. Ostsee*, Tl. 10, e 2, pp. 57–133 (Lf. 18).

Richardson, H., 1905. A monograph on the Isopods of North America. *Bull. U.S. nat. Mus.*, No. 54, 727 pp.

Stephenson, K., 1915. Isopoda, Tanaidacea, Cumacea, Amphipoda (excl. Hyperiidea). *Rep. Danish oceanogr. Exped. Medit.*, Vol. 2, No. 3, 1053 pp. Copenhagen.

Tattersall, W. M., 1906. The marine fauna of the coast of Ireland. Pt. 5, Isopoda. *Rep. Sea Inl. Fish. Ire., Sci. Invest.*, Year 1904, No. 2, 90 pp.

—— 1911. Die nordischen Isopoden. *Nord. Plankt.*, Abt. 6, pp. 181–313.

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RECEPTOR ELEMENTS IN THE COXAL REGION OF DECAPODA CRUSTACEA

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(Plates I and II; Text-figs. 1–8)

Investigation of the proprioceptive organs in the coxal region of crustaceans, the results of which are here recorded, have been to a large extent stimulated by the evidence put forward by physiologists. The probability of the occurrence of such organs across the thoracico-coxal articulations was pointed out by Prof. S. Dijkgraaf in 1955, in a letter to one of the authors (J.S.A.). During systematic study of sensory organs in the legs of crustaceans, made in the autumn of 1956 by the present writers, it was found that in the region of all articulations there are one or two sensory organs consisting of a connective tissue strand and a row of bipolar nerve cells. A paper by Dijkgraaf (1956*a*) on the compensatory eye movements in *Palinurus vulgaris*, in which it is concluded that some proprioceptors in the coxal region must be present, gave us the incentive to persist in examination of this region in spite of considerable technical difficulties. The results obtained fully confirmed Dijkgraaf's view and the presence of receptor organs in several species has now been established. It has been found, moreover, that in the coxal region they are of several kinds differing in their structure and arrangement from any receptor known as yet. The description of them is given below.

The investigations were made chiefly with the lobster, *Homarus vulgaris*, and the crabs *Maia squinado* and *Carcinus maenas*. Some observations were made on *Palinurus vulgaris*, *Astacus astacus*, *Eupagurus bernhardus* and *Cancer pagurus*. Methylene blue was used for staining. Usually the tissues were immersed in a weak solution made by adding 10–15 drops of 0·5% methylene blue in distilled water to 100 ml. of sea water. The nerves of the receptor organs stain in every such preparation provided they are well exposed, but this is a condition not easily fulfilled, as the organs are in a position not at all convenient for observation. Another procedure consisted in injecting a stronger solution of the dye (one part of the 0·5% solution mixed with three to six parts of sea water) directly into the region to be examined, before the animal was dissected. The results were not so consistent as with the immersion method, but sometimes gave better staining of the more deeply situated elements. Some hints on the dissection of the animals are given below (pp. 615 and 622).

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[1] The continuation of this work at the Plymouth Laboratory has been made possible by a grant from the Royal Society, for which the writer wishes to express his sincere gratitude.

After staining the preparations were fixed in ammonium molybdate, washed in water, dehydrated in absolute alcohol and mounted in xylol dammar.

OBSERVATIONS

Homarus vulgaris

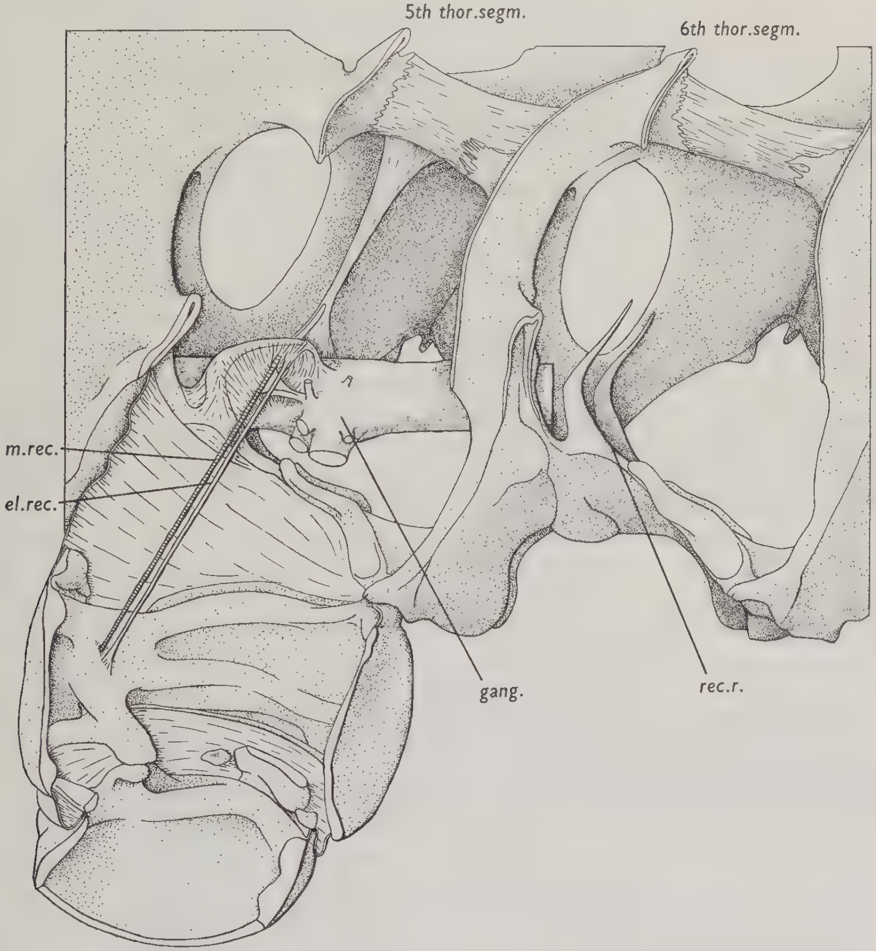
In the coxal region of *Homarus* the following nervous organs have been found: (1) receptors of the thoracico-coxal articulation; (2) receptor organ of the coxo-basipodite articulation; (3) a system of innervated elastic strands.

Receptor organs in the thoracico-coxal articulation

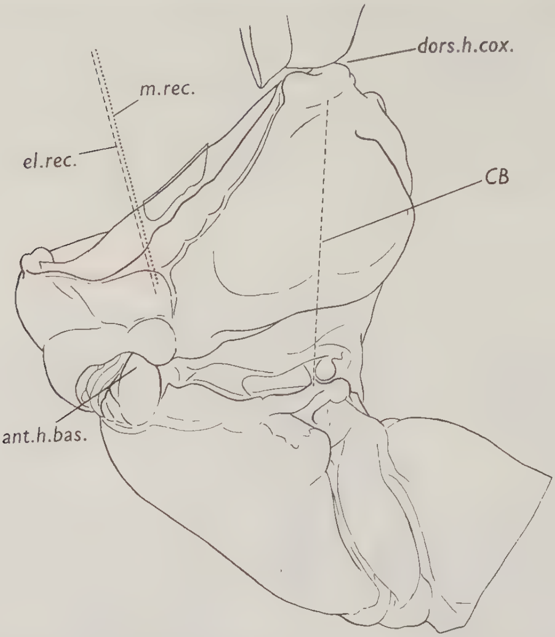
These receptors are present in all five peraeopods, in each of which there are two organs, a thin muscle with a special innervation and a strand of connective tissue with a number of sensory nerve cells. In the following description they will be termed the muscular and elastic receptors, respectively. These organs run side by side from their attachment on the endophragmal skeleton in the vicinity of the ganglion of the neural cord, to their insertion on the coxopodite (Text-fig. 1). In the 4th to 7th thoracic segments, that is those of the 1st to 4th peraeopods, the receptors originate on special projections of the endophragmal skeleton for which the term receptor rods is proposed. The rods arise close behind the ascending parts of the endosternites which, with their upper expansions, the mesophragms, form arches over the ganglionic cord and contribute to the walls of the sternal canal.

As seen in Text-fig. 1, the receptor rods are curved; their lower parts are directed upwards and forwards but then bend so that their upper parts are directed up with a backward inclination. Starting with a broader base at the border of the endosternites, they diminish in thickness and become slender and flexible. In the 4th thoracic segment the basal rigid portion is short, whereas the upper flexible part is long and has a strong backward inclination. In the following segments the rigid portions become gradually longer and the inclination of the upper parts less pronounced.

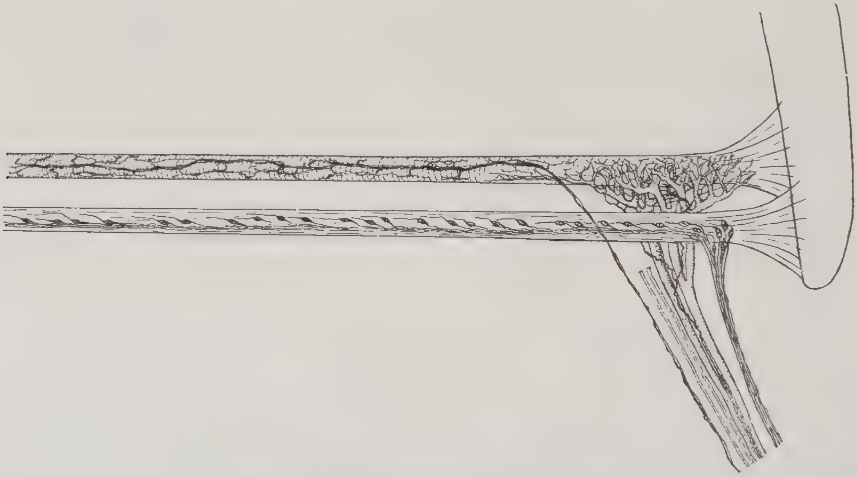
The receptor rods are enveloped by a connective tissue membrane which is continuous with the sheath of the neural cord. From the tips of the rods connective tissue strands extend upwards to the roof of the sternal canal where they blend with the membrane lining its walls; they help to keep the flexible parts of the rods straight in the position shown in Text-fig. 1 on the right side of the 5th segment, for when they are severed, as normally happens on dissection aimed at exposing the neural cord from the dorsal side, the rods assume the curved shape shown on the left side of the same segment. They straighten at once if their enveloping membrane is pulled away, as in Text-fig. 1 in the 6th segment; this often happens unintentionally, as the connexion of the membrane to the rods is very loose and they easily slip out of it. No reference to these structures of the skeleton could be found in the literature; they are represented in one figure in the work of Milne Edwards (1851, Pl. 9, fig. 3), but there is no mention of them in the text or in the explanation of figures.



Text-fig. 1. *Homarus vulgaris*. Parts of the 5th and 6th thoracic segments seen from the lateral side and slightly from behind. In the 6th segment the receptor rods, *rec.r.*, are shown freed from connective tissue. In the 5th segment the receptor rod of the right side is shown in its natural position, kept straight by a connective tissue strand; that of the left side is shown curved as it usually looks when exposed from above. *gang.*, ganglion of the neural cord; *m.rec.*, *el.rec.*, muscular and elastic receptors of the second peraeopod.



Text-fig. 2. *Homarus vulgaris*. Part of the left second peraeopod from in front and slightly laterally showing the projection of the distal attachments of the muscular (*m.rec.*) and elastic (*el.rec.*) receptors and of the position of the coxo-basipodite receptor, *CB*; *dors.h.cox.*, dorsal hinge of the coxa; *ant.h.bas.*, anterior hinge of the basipodite.



Text-fig. 3. *Homarus vulgaris*. Semi-diagrammatic drawing showing proximal parts of the muscular receptor (above) and the elastic receptor (below) with associated nerve elements. Nerve fibres of the additional innervated strands are drawn in dotted lines (cf. Text-fig. 5).

The two receptors are attached to the flexible part of the rod, the elastic receptor to its tip, and the muscular one not far from it (Text-fig. 3). Near the attachment of the muscular receptor its myofibrils are replaced by connective tissue fibres which thus serve as a short tendon; these fibres and those of the elastic receptor spread fan-wise in passing into the membrane around the rod. The two receptors run from their origin to their insertion close together without having any connexions with each other. A nerve given off by a neighbouring trunk squeezes its way between the receptors, but otherwise has not any relation with either of them.[1]

The distal attachments of the two receptors are not at the same spot. The point of insertion of the muscle on the coxopodite can best be determined in relation to the anterior hinge of the basipodite. If an imaginary line parallel to the axis of the leg be drawn through this hinge, the point of insertion of the receptor muscle would be found a little laterally to this line, and nearer to the proximal than the distal border of the coxopodite. On the inner surface of the coxa there is often a small depression marking this spot, the position of which, projected on to the outside, is shown in Text-fig. 2. It should be noted that here the muscle tissue of the receptor extends up to the cuticle. The elastic receptor inserts a little farther distally and medially from this point; its fibres pass into the membranous tissue of the dermis and since they diverge and blend with this membrane their exact endings cannot be defined as well as that of the muscle.

In their proximal parts the two receptors are of nearly the same calibre, measuring in middle-sized specimens about 90μ. The elastic receptor is of about the same thickness all along its length except at the very end, whereas the muscular one becomes gradually thicker distally.

In the 8th thoracic segment, where no special receptor rod is present, the two receptors originate on the pillar of the endosternite. Otherwise they exhibit the same features, a slight difference being that the elastic receptor is comparatively longer, so that its insertion is more distal.

Structure of the muscular receptor

The muscular receptor is composed of bundles of myofibrils with a small amount of connective tissue between them; stronger connective tissue fibres run longitudinally on its periphery. The cross-striation of the myofibrils is somewhat finer than in the neighbouring ordinary muscles, being of a similar type to that of one of the muscles (RM 1) of the abdominal receptor organs (Alexandrowicz, 1951).

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[1] This nerve, which arises from a trunk distributing its branches in the coxopodite, is peculiar in that it consists of bipolar nerve cells and their processes. The cells, which differ in their staining properties from the sensory elements of the receptors, are scattered along the nerve with two accumulations, one near the ganglion and the other at the diaphragm of the fracture plane of the leg. It is on this diaphragm that the distal cell processes, or at least the majority of them, end. Similar ganglionated trunks have been observed in *Palinurus* and in crabs. The occurrence of a nerve of such unusual structure in this part of the body suggests that it might play some role in the regeneration of an autotomized limb. Another interesting feature observed is the presence of a plexus of fine nerve fibres in the sheaths of the main trunks arising from the ganglion.

A broad bundle of nerve trunks extends between the ganglion and the muscular receptor (Text-fig. 3, Pl. I, figs. 1–4). Two sorts of nerve fibres can be distinguished: (1) motor fibres innervating the muscle tissue, and (2) fibres ending at the proximal end of the receptor in its tendinous region. The former run down alongside the muscle giving off branches which penetrate between the myofibril bundles, subdividing to supply the bundles abundantly up to the distal end of the muscle. This innervation has the same characteristic pattern as in all other muscle receptors, which is a great help in locating these organs among ordinary muscles.

The fibres ending in the tendinous region appear to be of two kinds. Most of them are given off by two stout main fibres which as they approach the receptor divide into a variable number of unusually thick branches; each of these breaks up into numerous arborizations which penetrate between the fibres of the tendon. Considering the thickness of the main trunks and the density of their ramifications it appears that the amount of nerve substance is here greater than that of connective tissue (Text-fig. 3; Pl. 1, figs. 3, 4). Some of the nerve branches extend into the region of the connective tissue which accompanies the muscle; one or two branches may be seen running some distance along the muscle, but there is no evidence of any anatomical relation between this sort of nerve and the muscle tissue itself.

The appearance and distribution of the thick trunks in the tendinous region of the muscular receptor is much like that of the dendritic processes of the nerve cells situated near the muscle components of the receptor organs of the extensor muscles of the thorax and abdomen. In the coxal receptors, however, no cells are present near the muscle. Consequently, the cells emitting these trunks must be situated in the ganglion of the neural cord, and, as there can be no reasonable doubt about the sensory nature of the fibres terminating in the tendon, they must be regarded as dendritic processes of receptor neurons.

The exact situation of the cell bodies of these neurons has not been established. The main difficulty is that the cells in the ganglion take a much longer time to stain with methylene blue than the fibres on the receptor, and when the latter are fading out their tracing up to their cells becomes uncertain. This obstacle might perhaps be more easily overcome in some other species.

Among the thick fibres reaching the receptor some others of a much finer calibre have been observed, which are presumably of a different kind. The number of these accessory fibres is uncertain, and as regards their destination it can only be said that they pass into the entanglements of the terminations of the thicker nerves.

Structure of the elastic receptor

The elastic receptor consists of longitudinally directed fibres forming a tube in which a row of nerve cells and their processes are included. The fibres have elastic properties, but must be made of some substance other than the elastin of vertebrate tissues, for they do not show a specific affinity to orcein or to Weigert's resorcin fuchsin. Stained with Azan they appear brilliant red, and so, in sections through their proximal attachments, stand out well against the blue-stained tissue of the membrane enveloping the receptor rods. They can be stained with iron haematoxylin and, more selectively, with Gomori's method. The latter proved useful for obtaining a contrasting coloration of these fibres and the muscle tissue. It should be noted that the fibres on the periphery of the receptor muscle, and in other organs described below, show the same staining reaction.

The nerve cells form a row which extends almost the whole length of the receptor. They are all bipolar in shape but vary in their dimensions. The largest are situated near the proximal end of the receptor, where some may lie outside the strand. The size of the cells diminishes towards the distal end of the strand; finally they become so small that often there is doubt whether a swelling of the nerve fibre is a cell or a bead-like artifact. It is for this reason, and also because not all the cells in the tube stain equally well, that their number is uncertain; it appears to exceed fifty, but may be considerably greater. The distal processes of the cells, which are often of a wavy appearance, are applied to the fibres of the strand and evidently become attached to them, as they all keep the same direction parallel to the axis of the organ. If the axis of a cell body is not in line with its process, the latter, in order to become adjusted to the longitudinal direction of the elastic fibres, turns at an angle which, at the proximal end of the cell row, can even be acute.

The distal processes can be followed for a distance of about two or three lengths of the cell bodies, at which point the methylene-blue coloration usually stops. In some preparations a finer prolongation beyond this point may be noticed, but it is doubtful whether it is to be regarded as consisting of nervous substance. The proximal cell processes, the axons, run as a bundle alongside the cell row, enclosed within the same tube of elastic fibres. As each cell adds its axon to it, this bundle becomes thicker proximally. Coming out of the receptor, the nerve bundle runs towards the ganglion near to that of the muscular receptor (Pl. I, fig. 2).

The receptor of the coxo-basipodite articulation

The receptor of the coxo-basipodite articulation belongs to the system of similar organs occurring in all joints of the peraeopods of decapods, from the coxopodite downwards. It consists, as do all others of this system, of a connective tissue strand and a row of bipolar nerve cells. It is situated on the triangular plate of the coxopodite, the apex of which is the dorsal hinge articulating with the thoracic skeleton. The connective tissue fibres of the receptor originate near this hinge and run downwards to the rim of the basipodite near the insertion of the m. levator basipoditis, that is, at a point about halfway between the anterior and posterior hinges of the basipodite. The position of this point projected on to the outside of the leg is shown in Text-fig. 2.

The row of nerve cells begins at a certain distance from the proximal end of the organ; numerous nerve cells are accumulated here, many of them lying outside the strand (Pl. I, fig. 6). Farther distally they form a more regular row and become gradually smaller until they are minute. In the proximal accumulation both larger and smaller elements are present, but they are never as small as those at the distal end of the strand. The distal processes of the cells are applied to the fibres of the strand in the same way as in the elastic receptor previously described. In short, the structure of these organs is fundamentally the same, the difference in the appearance of the coxo-basipodite receptor being due only to features of secondary importance such as the less regular arrangement of its fibres and of the nerve cells.

Owing to the shape of the coxopodite its dorsal hinge is situated more proximally than the point of insertion of the two receptors of the thoracico-coxal articulation, which consequently extend distally beyond the point of origin of the coxo-basipodite receptor. This arrangement is shown in Text-figs. 2 and 4.

The system of innervated elastic strands

In this system which is regarded as constituting the receptor elements of m. levator basipoditis and m. depressor basipoditis the following parts can be distinguished: (1) a stronger non-innervated strand which will be referred to as suspensory ligament, (2) the main innervated strands, and (3) the additional innervated strands (Text-figs. 4 and 5).

Suspensory ligament

The suspensory ligament is a flat band of connective tissue which extends from underneath the neural cord towards the dorsal hinge of the coxopodite, dividing into branches which insert on several points situated at various distances from the hinge. The mesial part of the ligament is continuous with a membrane lying under the ganglion, which has a firm attachment to the borders of the endosternite, at the place where the short heads of the m. levator and m. depressor basipoditis originate.[1] From this point the suspensory

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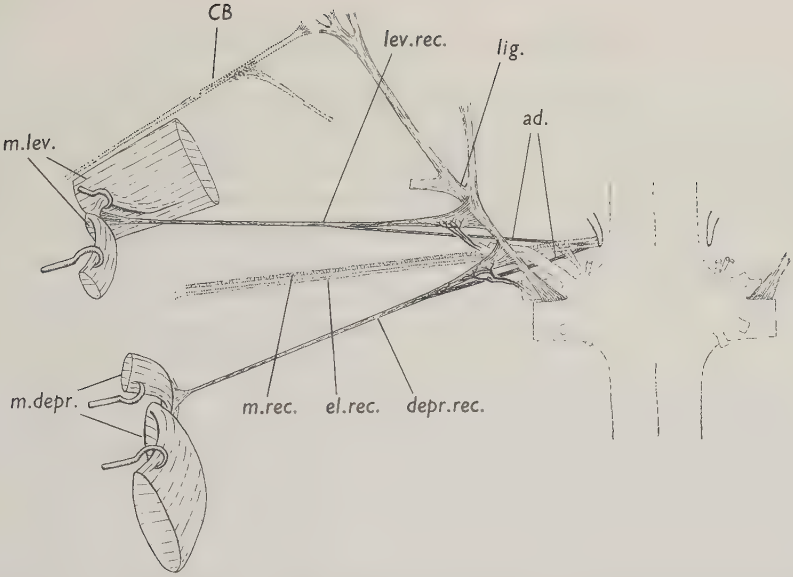
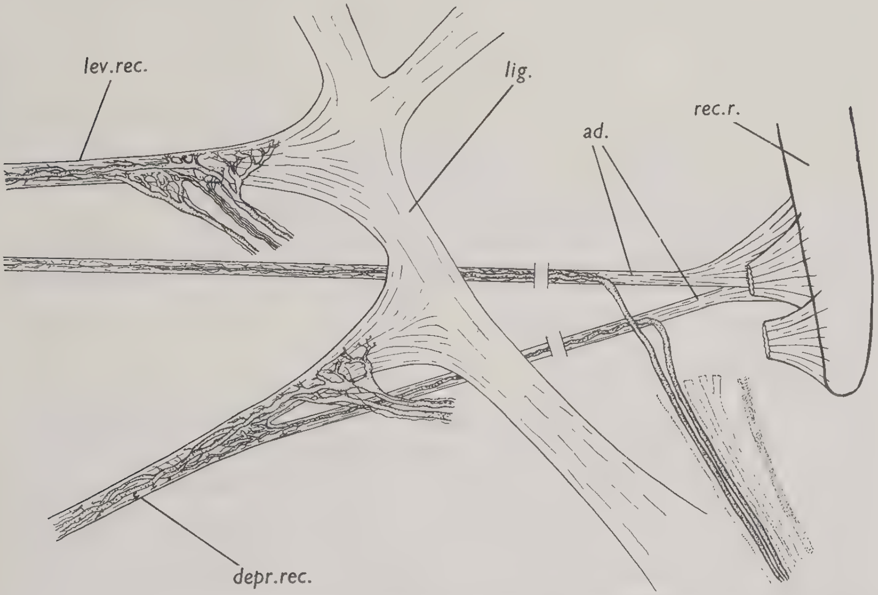
[1] These portions of the named muscles are situated beneath the main nerve trunks arising from the ganglion. According to Schmidt (1915) they originate, in *Astacus*, at the proximal border of the coxopodite. In *Homarus* their line of attachment has been found more proximally, along the border of the endosternite. The nomenclature of these and other muscles mentioned in this paper is that of Schmidt, which has also been adopted by Cochran (1935) and by Balss (1941).



Text-fig. 4. *Homarus vulgaris*. Topography of the system of innervated elastic strands in relation to other receptors in the coxa. The elements represented as if lying in one plane are in the animal body arranged in a cylindrical fashion, those seen in the upper part of the figure being approximately opposite to those in the lower part; besides, the axis of the coxa is almost perpendicular to the horizontal plane of the neural cord. *lig.*, suspensory ligament; *lev.rec.*, *depr.rec.*, levator and depressor receptors; *m.rec.*, *el.rec.*, muscular and elastic receptors; *CB*, coxo-basipodite receptor; *m.lev.*, *m.depr.*, parts of the levator and depressor muscles; *ad.*, additional innervated strands.



Text-fig. 5. *Homarus vulgaris*. Nerves of the levator and depressor receptors; same lettering as above. Note the difference in the innervation of each strand. The muscular and elastic receptors are cut away near their origin at the receptor rod (*rec.r.*) (cf. Text-fig. 3).

ligament passes between the nerve trunks arising from the ganglion, just in front of the thickest of them, and running outwards divides into three main branches. One branch crosses the long head of the levator muscle on its inside (being here covered by some bundles of the muscle) and inserts on the coxopodite near the dorsal hinge by several diverging strands which pass into the dermis; one of these strands ends close to the proximal end of the coxo-basipodite receptor. A second, shorter, branch of the suspensory ligament inserts on the ventral border of the endopleural plate, not far from the dorsal hinge of the coxa. The third, which is longer, is attached to the coxa near the origin of the epipodite. Each of these main branches may give off strands securing additional attachments. The arrangement of all these parts is not exactly the same in different segments, those of the 5th leg especially showing major differences.

The suspensory ligament consists of fibres giving the staining reactions of collagen.

Main innervated strands

There are two main innervated strands connecting with the suspensory ligament by means of diverging fibres (Text-figs. 4, 5; Pl. II, fig. 7). One of the strands originates at a point on the ligament situated anteriorly to the thickest nerve trunk of the segment and runs between the short and long heads of the m. levator basipoditis towards their distal ends, where it penetrates between the bundles of the long head and splits into several very thin filaments which end between the insertions of the muscle fibres (Text-fig. 4, *lev.rec.*).

The second main strand has its origin at a point situated ventrally and medially to that of the first. From here it passes to the outside of the short head of the m. depressor basipoditis and in its further course runs between this muscle and the integument, in a direction slightly oblique to that of the muscle fibres. Without penetrating between the muscle bundles it terminates on the connective tissue near the insertion of the muscle (Text-fig. 4, *depr.rec.*).

Additional innervated strands

The two additional strands are attached to the receptor rods of the muscular and elastic receptors in the 4th to 7th thoracic segments, and in the 8th to the pillar of the endosternite. They run at an acute angle to the receptors and to each other; each becomes fused with one of the main strands, at points some distance from the suspensory ligament. The main strand ending with the levator muscle receives its additional strand about half-way along its length, but the second additional strand is shorter and reaches the main depressor strand nearer the ligament (Text-fig. 4).

The innervated strands consist of fibres of elastic properties, for they can be stretched to about twice their length and return to the original condition when the extending force is relaxed. Evidently they are made of the same substance as the fibres of the elastic receptor. In sections through the region of their attachment to the ligament, stained with Azan, the passage of the thicker red fibres of the elastic strands into the blue-stained finer elements of the ligament can be well observed.

Nerves of the innervated strands

The arrangement of the nerve fibres in the innervated strands shows particular features in each of them (Text-fig. 5).

The strand inserting at the depressor muscle, which for short is termed the depressor receptor, receives three stout fibres. Two of them come from the nerve trunk arising next in front of the thickest trunk of the leg. Along with these stout fibres run a few others, of extremely fine calibre, the number of which is difficult to determine. A third stout fibre, but not so thick as the two others, travels in the additional strand. It comes out of the ganglion with the bundle of nerves of the muscular receptor, penetrates into the additional strand and reaches the main strand near the other nerves and distally to them. All three fibres break up into numerous branches, their terminations supplying the strand in a good part of its length (Pl. II, fig. 8).

The other main strand, the levator receptor, receives: (1) two stout fibres, associated with very thin ones as in the other strand; (2) a bundle of several fibres, probably six, which are much thinner, but not so thin as those accompanying the two stout ones. All these fibres are given off by the nerve trunk which originates from the ganglion with a common root with that containing the nerves to the depressor strand, but from a more dorsal division of it which carries motor and sensory nerves of the coxopodite.

The additional strand of the levator receptor receives its nerve fibre from the ganglion in the same bundle as that of the other additional strand (Text-fig. 5). Penetrating into the strand, this nerve gives off many ramifications ending on the elastic fibres (Pl. I, fig. 5); distally the nerve elements become gradually finer and less distinguishable. If some of them pass on to the strand they can be only very thin ones.

To sum up: both the depressor and levator receptors have a similar nerve supply of two stout fibres accompanied by very fine ones. They differ in that the depressor receptor gets a third thick fibre brought by its additional strand, whereas the levator receptor receives a bundle of thinner fibres from the same nerve trunk in which its two thick fibres are travelling. As to the additional strands, one appears only to carry a third fibre to the depressor receptor, whilst the second is an organ in its own right with nerve terminations on its elastic components.

As regards the origin of the nerve fibres it has been noticed that the two thick fibres of the depressor strand, when followed in the proximal direction, are found at the ganglion close to the bundle containing the fibres of the muscular receptor and those of the additional strands. It is possible that the nerves of the levator strand have a similar origin, but this could not be observed directly with sufficient certainty.

Although so many nerve elements are mentioned above, the description may not yet be complete. In some cases, for instance, thin fibres have been noticed which possibly enter the additional strands with the thick ones, but they were not distinct enough to be followed.

The relation of the nerve elements to the strands is interesting in several points. One is the calibre of the nerve fibres, which is larger than is ever met with in nerves supplying a muscle bundle of comparable dimensions. Another is the tendency of even the thick nerves to penetrate between the elastic fibres of the strands and to occupy an axial position; this feature can best be observed in transverse sections. In the main strands there is a somewhat irregular intermingling of various elements, but in the additional levator strand it can clearly be seen that the elastic fibres form a tube in which the nerve fibre and its branches are enclosed.

The branches of the nerve fibres in the main strands are of various lengths and thicknesses. In the region where the main fibres enter the strand they give off many thick short branches, the ramifications of which end after a short course (Pl. II, fig. 8). The picture of their terminations in methylene-blue preparations is hazy, but from what can be seen it can be inferred that the area of distribution of each branch is small. As, however, these branches are numerous it looks as if nerve endings were present at every point of the elastic fibres, as far as the nerves reach. The thin short branches of the main trunks behave like the secondary branches of the thick ones. The long branches run down the strand and give off in their turn shorter ramifications ending near their origins and longer ones continuing the distal course. It is not quite clear how far the nerves extend in each of the strands, but it may be assumed that they occupy at least the half of their length. It has not been possible to determine whether or not each of the main fibres sends its branches as far as the others. The best preparations show such an abundance of intermingling nerves that the tracing of individual elements is uncertain.

As there are no cells between the endings of these fibres and the ganglion it is evident that the cell bodies of all these neurons lie within the central nervous system. Since there can hardly be any doubt that the innervated strands having relations with muscles are receptors of some kind, their nerves, at least the majority of them, have to be regarded as afferent fibres, being elongated dendritic processes of sensory nerve cells. The nature of the thin fibres is an open question as other interpretations are possible.

Hints on dissection

All parts of the animal body should be cut away leaving only the ventral part of the thorax with the coxopodites and parts of the basipodites. The sternal canal should be opened from above, beginning at the posterior segments, and the parts of the endophragmal skeleton cut down to a level slightly above the dorsal hinge on the coxa. The receptor rods can now be located more or less easily depending on the size of the specimen; they appear curved as shown in Text-fig. 1 if the membrane enveloping them has not been damaged. To expose the muscular and the elastic receptors in their whole length it is necessary to remove a good part of the chitinous case and of the muscles of the coxa, while it is borne in mind that the receptors run almost vertically from their origin on the rods towards the anterior hinge of the basipodite; the lines of incisions should therefore run down the coxa one a little in front of its dorsal hinge, and the other on the opposite side of the leg. It is better not to try to expose the receptors at once, but to put the preparation into the staining solution, and to continue the dissection after the proximal parts of the receptors become visible. The staining is often impeded by clots of blood adhering to the receptors and other organs; the clots can be removed with a forceps and a fine scalpel, but to do this without spoiling the organs is a delicate operation. Two ways of proceeding further can be followed. The preparations can either be left in the staining solution and then fixed without removing the chitin, which goes off easily later after fixation in ammonium molybdate, or the soft parts can be detached from the chitinous cuticle at this earlier stage. In the latter case the receptor rods must be cut through first, and then all soft parts cautiously separated from the cuticle, while the muscle tendons should be cut through at their bases. Then the preparation can be spread and pinned on to a paraffin plate. This method has the advantage of permitting better observation of the staining, but the tissues are easily damaged.

To obtain preparations with the suspensory ligament and the coxo-basipodite receptor, the lateral line of incision must be made behind the dorsal hinge of the coxa. It is possible thus to get all receptor organs on one slide. For study of innervated strands it is preferable to remove the chitin during the staining because otherwise it is difficult to spot the strands and expose them in the right way.

The only certain way of finding the suspensory ligament is by tracing the innervated strands up to their origin on this ligament, but the strands cannot be practically identified until their nerves have stained. The separation of the ligament from the arteries, nerves, connective tissue strands and blood clots is difficult, and can best be performed after the preparations have been transferred into xylol.

BRACHYURA

Proprioceptors originating at the endophragmal skeleton and ending on the coxopodites have been found in all the crabs investigated, but their arrangement is not the same as in *Homarus*, the important difference being that the elastic receptor appears to be missing. The innervated strands are present in *Cancer* and in *Carcinus*, though forming a simpler system than that of the lobster; in *Maia* they could not be found as separate organs.

Muscular receptor

The muscular receptors are present in all five peraeopod segments, but only in that of the chela is there a special chitinous projection to which the muscle is attached and for which the term receptor rod may appropriately be used. No mention of this part of the skeleton could be found in the literature, so it will be described here.

The receptor rod is a long thin projection from that plate which limits the 4th thoracic segment anteriorly, and which is called the second endopleurite by Drach (1939).[1] The receptor rod arises from the ventral border of this plate, laterally to the mid-point of the border, and projects at an acute angle inwards and backwards, ending at approximately the same distance from the mid-line of the body as the inner corner of the endopleurite plate (Drach's apophyse interne) (Text-fig. 6, *rec.r.*). The length of the rod in examples of the three species was as follows:

	Length and breadth of carapace (cm)	Length of rod (mm)
Maia	14 × 12	11·5
Cancer	10 × 15	17
Carcinus	5·5 × 7	10

In *Maia* the rod is comparatively shorter; it arises at a less acute angle and its tip is situated more behind and farther away from the corner of the endopleural plate than in the other species. The tip of the receptor rod is attached by feeble connective tissue to the ligament extending from this corner to the next-following arthrophragm. The point of attachment lies about half-way along this ligament (Text-fig. 6).

In *Cancer* and in *Carcinus* the rods project at a more acute angle from the edges of the endopleural plates, close beneath them. As the endopleural plates and the receptor rods are prolonged farther towards the mid-line of the body than in *Maia*, only a small distance separates the ends of the rods of the opposite sides. The ligamentous connexions between the corners of the endopleural plates of the two sides and between these plates and the endosternites are stronger than in *Maia*; in *Carcinus* there are also short muscles here.

In *Maia* the receptor rod is thickened at its terminal part and is slightly bent (Pl. II, fig. 10). In *Carcinus* it may be almost straight as in *Maia*, or there may be a more pronounced bend at the tip (Pl. II, fig. 9). In *Cancer* the rod curves and tapers into a point. In all these crabs the muscular receptor originates near the tip of the rod and runs at an acute angle to it to insert on the rim of the coxa, near the ventral border of the tendon of the m. promotor coxopoditis; in its course it passes on the ventral side of this muscle, between it and the m. depressor basipoditis.

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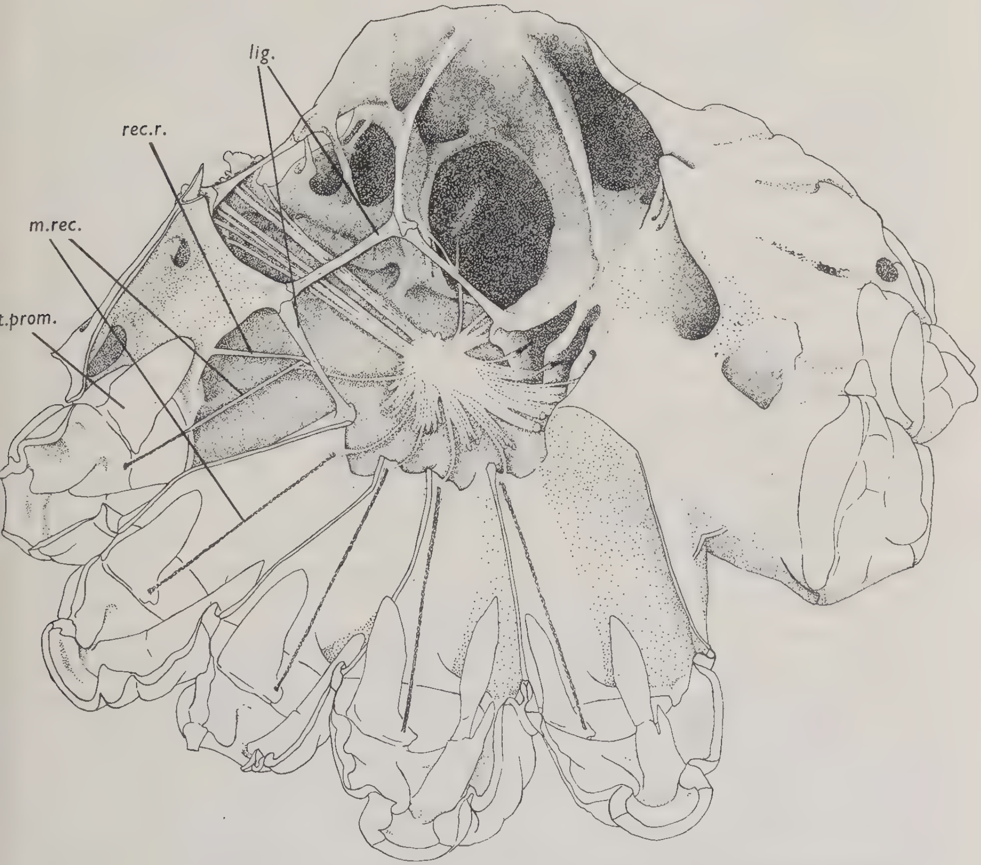
[1] Pearson, in his monograph of *Cancer* (1908) designates this plate as the endosternite of the 8th somite, that is, of the 4th thoracic segment. Drach's denomination has been adopted here, although without knowledge of the development of this arthrophragm there is no certainty that the lower part of the plate in question is not derived from the lateral part of the sternum. The number (2nd) of this endopleurite is due to the fact, known since the work of Milne Edwards (1834, 1851), that each endopleurite fuses with the endosternite of the following segment and not with that of its own. To be exact, they do not fuse directly but join to form a horizontal plate (lame de jonction of Drach). The ventral border of the 2nd endopleurite plate is free since the other part of the arthrophragm, an endosternite plate ascending from the sternum and fusing with the endopleurite plate, is here missing.

In the 5th to 8th thoracic segments, in which no receptor rods are present, the muscular receptors originate directly on the posterior surface of the endosternites close to their mesial border at a level higher than that of the dorsal surface of the ganglionic mass (Text-fig. 6). From this point of attachment the receptor in each segment runs between the promotor and depressor muscles on the posterior surface of the endosternite, being separated from the chitin of this plate only by a thin layer of the fibres of the m. promotor (Text-fig. 7).



Text-fig. 6. *Maia squinado*. Endophragmal skeleton with parts of the peraeopods dissected on the left side to show the muscular receptors (*m.rec.*). *rec.r.*, receptor rod; *t.prom.*, tendon of m. promotor coxopoditis; *lig.*, ligaments of the 2nd endopleurite.

The distal attachments of the receptors in the 2nd to 5th peraeopods are not exactly at the same points in various species. In *Cancer* they insert, as in the chela, on the rim of the coxa close to the base of the promotor tendon, but in *Maia* and *Carcinus* they insert in the base of the tendon itself. The arrangement of the receptors in *Maia* is shown in Text-fig. 6. At the distal insertion the muscle tissue extends up to the chitin, but at the proximal end of the receptor its myofibril bundles are fixed to the chitin by a short tendon of connective tissue fibres about 100–200 μ long (Text-fig. 8, *tend.*).

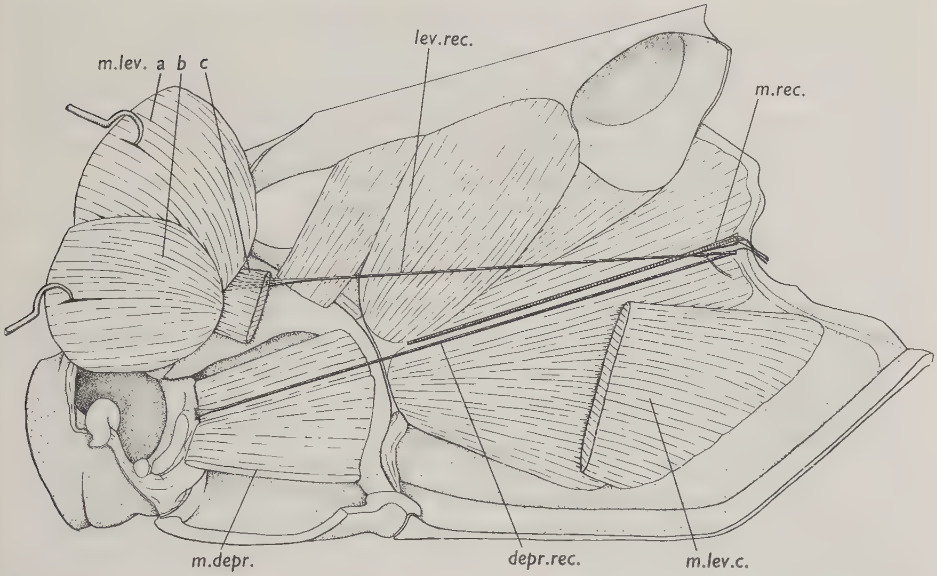
Apart from having a special rod for its attachment the structure and innervation of the receptor rod of the chela does not show marked differences from those of the other legs, and the description given applies to the organs of all five peraeopods.

The muscle of the receptor is surrounded by longitudinal connective tissue fibres which run down to its insertion. At its proximal end there are, besides, two strands of connective tissue attached to the chitin near the origin of the muscle, and flanking it on the dorsal and ventral side. In their distal course they come to occupy more and more of the periphery of the muscle, blending with the fibres mentioned above, until it is surrounded evenly by a sheath of longitudinal fibres. In *Carcinus* and *Cancer* the strands are of about the same width near the origin of the muscle (Text-fig. 8). They enclose nerve fibres and their apparent thickness is mainly due to the large calibre of the nerve trunks within them. In *Maia* the ventral strand is very wide and contains nerve fibres also, while the dorsal one, in which no nerve fibre is enclosed, is much narrower (Pl. II, fig. 12).

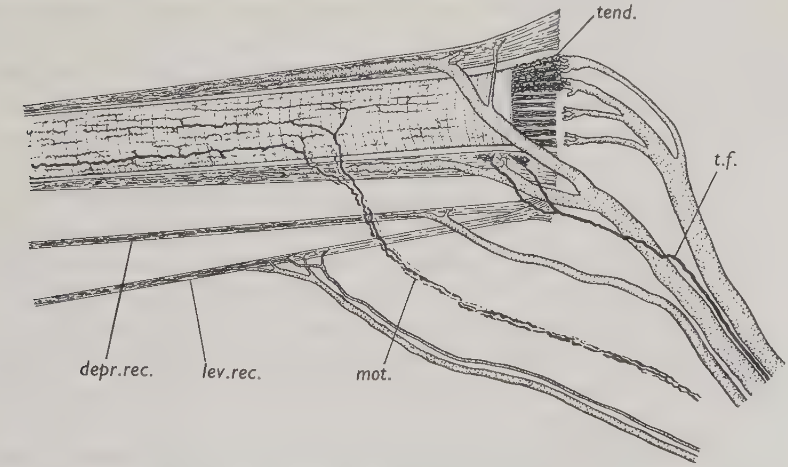
The muscular receptors have two sorts of nerves, motor fibres for the muscle tissue and fibres ending on the connective tissue associated with the muscle. The arrangement of the nerves shows some differences in the species investigated.

In *Carcinus* (Text-fig. 8) all the nerve fibres reach the receptor near its origin, but the motor fibres travel from the ganglion in a separate trunk. The distribution of their divisions and subdivisions on the muscle tissue has the same pattern as in *Homarus*. The nerves of the sensory apparatus approach the proximal end of the receptor as a bundle containing three thick and one, perhaps more, thin fibres. Followed back to the ganglion this bundle is found running along the nerves of the limb, without uniting with them firmly as a common trunk; it arises on the dorsal surface of the ganglion anteriorly to the other nerves of the same segment. In the 4th segment it has to travel upwards to reach the muscle arising on the receptor rod.

One of the three thick fibres approaches the receptor directly opposite its end and breaks up into several stout branches which pass on to the short tendon of the muscle and supply it with dense ramifications. In methylene-blue preparations little can be seen of their finest divisions, but it can safely be assumed that they end among the elements of the tendon. One or two nerve branches can sometimes be seen extending a little farther, between the bundles of myofibrils, but no relation to the latter has been noticed.



Text-fig. 7. *Carcinus maenas.* Posterior view of the endosternite separating the 5th and the 6th thoracic segments of the left side with parts of the coxa and the basipodite of the 3rd peraeopod. *m.rec.*, muscular receptor; *lev.rec.*, *depr.rec.*, levator and depressor receptors; *m.depr.*, short head of m. depressor basipoditis; *m.lev. a, b, c*, portions of m. levator basipoditis. The muscle bundles, the middle part of which is cut away (*m.lev. c*), originate, as shown in the figure, on the ventral part of the endosternite plate; they can be regarded as a branch of the strong head of the levator muscle (*m.lev. b*) which originates on the anterior surface of the endosternite of the next following segment (not seen in the figure).



Text-fig. 8. *Carcinus maenas.* Proximal parts of the thoracico-coxal receptors. The semi-diagrammatic representation applies to each of the 4th to 8th thoracic segments of the left side. The receptor muscle is seen flanked by two strands of connective tissue into which penetrate branches of a thick nerve fibre. *tend.*, tendon of the receptor muscle; branches of the nerve fibre ending on it have been partly cut to show the fibres of the tendon drawn somewhat diagrammatically. *t.f.*, thin fibre ending on the ventral strand; *mot.*, motor nerves of the receptor muscle. *lev.rec.*, *depr.rec.*, levator and depressor receptors with their innervation. For the sake of clarity the levator depressor is drawn in a position different from that in Text-fig. 7.

The second thick fibre bifurcates at the origin of the muscle; the two branches pass to either side of the muscle, where each enters a connective tissue strand and runs in it giving off both long branches, which arise at acute angles and continue the course in the strand, and short twigs starting at various angles and terminating on the fibres of the strand. It is difficult to assess how far down the muscle the nerves reach, for the branches are less and less distinguishable as they become finer. It appears that they run for a distance of from one-third to one-half of the length of the muscle.

The thin fibre often stains quite well, and there is ground for assuming that the methylene-blue preparations give a true picture of its share in the innervation of the receptor, although its behaviour appears strange. This fibre may be undivided, or may give off one or two branches. In either case the fibres end in a tuft of short branches occupying a small area on the strand flanking the muscle on its ventral side (Text-fig. 8, *t.f.*).

In the receptor of the chela, which otherwise does not show any difference in its innervation, thin filaments of very small calibre have been observed intermingling with the stout fibres. There is some indication that they are present also in other segments, but this could not be established with sufficient certainty.

The third of the thick fibres approaching the receptor is the element of an innervated elastic strand. There are two such strands, both originating near to the proximal end of the receptor (Text-fig. 8, Pl. II, fig. 11). One of them runs near and parallel to the receptor muscle, but extends farther, to become applied to the bundles of a short muscle stretching between the coxa and the basipodite and to end near to its insertion. According to Cochran (1935) this muscle may be regarded as a portion of the m. depressor basipoditis, so that the innervated strand under consideration may be called the depressor receptor (Text-fig. 7, *depr.rec.*).

The second innervated strand runs in the same direction as the first, at a very acute angle to it. Approaching the overlying bundles of the most posterior of the heads of m. levator basipoditis it runs alongside them and, splitting into very fine filaments, ends between the muscle fibres at their insertion into the basipodite (Text-fig. 7, *lev.rec.*). Because of its association with the levator muscle this strand may be called the levator receptor.

The nerve of the depressor strand is, as mentioned above, the third of the thick fibres running towards the muscular receptor (Text-fig. 8). It penetrates into the strand and behaves in a way similar to that of the nerves in the strands flanking the receptor muscle.

The levator receptor receives its nerves from one of the trunks running from the ganglion to the limbs. Two comparatively thick fibres reach the strand at a certain distance from its origin and give off branches which divide in their turn (Text-fig. 8, Pl. II, fig. 11).

Compared with the elements of the system of innervated strands in *Homarus* those in *Carcinus* show a simpler arrangement as there are two strands only, and the suspensory ligament is missing. As regards their innervation, however, it would be premature to draw a similar conclusion for the technical difficulties are such that other elements may yet be detected. It is also understandable that no details can be given about the behaviour of the various nerve elements in these strands, the dimensions of which may go down to 10 μ and in the thickest parts are in the range of 50–60 μ.

In the 4th thoracic segment the innervated strands originate on the receptor rods close to the attachment of the muscle and in all probability their distal insertions are the same as in other segments, but the observations have been hampered by the difficulty of obtaining preparations with these organs intact along their whole length.

In *Cancer* the arrangement of various receptor elements is similar to that in *Carcinus*. The innervated strands are also present. *Cancer* was less thoroughly examined than *Carcinus*, the latter being, despite its smaller size, more suitable for dissection.

In *Maia* the independence of the motor and sensory nerves of the muscular receptor is particularly manifest as the motor fibres run with the nerves of the promotor and depressor muscles, and reach the receptor at a distance from its proximal end. Two fibres at least supply the receptor, and it is interesting to note that one of these is a branch of a fibre which also supplies the promotor muscle. Reaching the receptor the motor fibres bifurcate and their branches run in opposite directions, ramifying in the usual way.

As in *Carcinus*, three thick and at least one thin fibre approach the receptor muscle at its origin. One of the thick fibres is destined for the short tendon and its situation is the same as previously described (Pl. II, fig. 12). The two others penetrate into the connective tissue strand flanking the muscle on its ventral side and branch in this strand in the same manner as in *Carcinus*.

The thin fibre runs together with the thick ones. It gives off short branches, all of which appear to end on the connective tissue strand at its proximal end. This fibre did not stain so well in *Maia* as in *Carcinus* and the picture of its course was not so clear.

In the receptor of the chela the arrangement of the three thick fibres is basically the same, although at first sight it may seem to be different (Pl. II, fig. 10). This is due to the fact that the two thick fibres penetrating the connective tissue strand can split into branches before reaching the receptor, and these branches enter the strand at a distance one from another. The very thin fibres running with the thick ones occur in *Maia*, too. They appear to associate with the thick fibre which supplies the short tendon of the muscle.

The innervated strands have not been found in *Maia*. It is, of course, possible that if they are in a different position they might easily be overlooked. However, the occurrence in *Maia* of the same number of thick nerve fibres, and the fact that one of them supplies the depressor receptor in *Carcinus*, gives probability to the supposition that the system of innervated strands in *Maia* has been reduced and one of its elements has become associated with the muscular receptor. A comparative study of these organs in various species of Brachyura could possibly throw some light on this problem.

In all crabs investigated a coxo-basipodite receptor, with bipolar nerve cells, is present. Owing to the different shape of the coxa in crabs, the position of this receptor is relatively more distal than in *Homarus* and does not come into the picture in preparations of the muscular receptor.

Hints on dissection

With crabs the main part of the dissection aimed at exposing the receptors consists in carefully cleaning away the liver; particular caution is necessary with regard to the organ in the 4th thoracic segment where it is attached to the receptor rod.

In *Maia* this rod can be found more easily from the dorsal side. On removing the liver piece by piece one finds a flaccid ligament connecting the endopleurites of the opposite sides (Text-fig. 6, *lig.*) and this guides one to the inner corners of these plates. This region must be cleaned with care in order not to damage the other ligament running backwards from the corner of the endopleurite plate on which the tip of the receptor rod is attached.

In *Carcinus* and *Cancer* it is preferable to look for the receptor rod from below in order to find its origin on the ventral border of the 2nd endopleurite. To make the access easier, parts of the sternum should be removed as far as possible, and the liver strands taken out. It must be realized that the receptor rod is very fragile and can be broken at a slight pull of the forceps. Its origin can be found on the border of the endopleurite plate at a point more laterally than in *Maia*. On moving the rod with fine forceps one can locate the point of its tip at which the receptor muscle is attached. The preparations of the rods with the receptors can be made by cutting out the rods with all the adjacent parts, i.e. muscles, nerves, remains of the liver, etc. The whole is put into methylene-blue solution and the tissues sorted out under a dissecting microscope. The rod with the receptor can be thus isolated and attached with fine pins to the paraffin plate on which it remains until the preparation is to be put into xylol.

The receptors in other segments can be stained *in situ*, but then only their proximal parts on the median borders of the endosternites can be made visible. For detailed study each endosternite should be cut out with parts of the coxa and the basipodite as in the preparation shown in Text-fig. 7. This is a simple matter, but there is one vulnerable point, viz. the connexion of the nerves with the receptor, and the utmost care must be taken in avoiding any pulling on these nerves. Therefore, before cutting through the sternites, it is important to sever the nerves, vessels and each strand which might tear the nerves. After the endosternite plate has been cut out it should be pinned on to a paraffin plate with its posterior surface upwards. The muscle bundles covering the receptor should be pulled aside and pinned down, but this must be done progressively. Little practice is necessary to recognize the receptor if only a small portion of it becomes stained; it can then be safely freed from overlying tissues. In every case of doubt about its further course the preparations should be put back into staining solution and examined after a while.

The innervated strand of the depressor receptor in *Carcinus* and *Cancer*, running parallel to the receptor muscle, can be found without difficulty, provided one does not cut the tissues around this muscle too freely at the beginning of the staining, because the fine innervated strand cannot then be distinguished and may be torn away.

The levator receptor can be found by pulling cautiously aside the bundles of the levator muscle. Here again it is advisable to do this by steps, proceeding each time as far as the course of the strand can be traced and leaving the preparation in the staining solution until it is discernible.

Some observations on other species

A few observations were made with *Astacus astacus*, *Palinurus vulgaris* and *Eupagurus bernhardus*; in each of these the receptor organs in the coxal region have been found. Although they have not been investigated in detail, some of their features are worth mentioning since they show that the anatomy of the receptors varies in different species.

In *Astacus* the general arrangement of the receptors is similar to that of *Homarus*. Receptor rods are present in the 4th to 8th thoracic segments; they arise from the bases of the pillars of the endosternites; in *Astacus*, as the sternites of these segments become progressively broader posteriorly the rods stand farther away from the nerve cord; there is no rigid connexion between the sternites of the 7th and 8th segments, and here the rods arise, not from the hard part of the endosternite, but from the arthrodial membrane behind it. As far as they have been determined, the structure and innervation of the muscular receptor, the elastic receptor and the coxo-basipodite receptor, resemble those of *Homarus*. Because of the more lateral position of the rods in the hinder segments, the nerves of the receptors are correspondingly longer in these segments. The suspensory ligament and elastic strands are again similar to those of the lobster, but with differences in the details of the arrangement and innervation of the elastic strands.

In *Palinurus* the receptor rods are present in all five thoracic segments, but their disposition in conformity with differences in structure of the endophragmal skeleton is not the same as in *Homarus*. It is only in the 4th thoracic segment that the endosternites arise near the median line of the body and have the receptor rods originating at their bases; in this segment *Palinurus* has long rods extending upwards and backwards as far as the roof of the sternal canal. In the 5th to 8th thoracic segments, in which the basal parts of the endosternites are situated at a considerable distance from the mid-line of the body, the receptor rods project from the upper parts of the endosternites, not far from the horizontal plates of the mesophragms; they originate on the posterior surface of the endosternite plates near their inner border, and are directed inwards. In all segments the rods are thin and very flexible.

The muscular receptor attaches to the rod, but the elastic receptor appears to be missing. At any rate it is not present in the immediate vicinity of the muscular one. A coxo-basipodite receptor is present. There are some strands of connective tissue which presumably correspond to the innervated elastic strands of *Homarus*, but their extension and connexions have not been sufficiently determined. *Palinurus* proved a difficult object for this kind of investigation, for in the specimens dissected there was soft yellowish tissue filling up the sternal canal and enveloping the outgoing nerve trunks; it made it difficult to find the receptors and prevented the stain penetrating.

In *Eupagurus bernhardus* the receptor rods are missing, so that the muscular receptors, the only ones which have been found, arise directly on the endosternites. They insert near the rim of the coxa.

DISCUSSION

The receptor organs described are remarkable in many respects. Not only are several of them present near to one another, but their structures show essential differences within the Decapoda. Thus in *Homarus* there are (1) an elastic strand with numerous nerve cells attached to it, (2) a thin muscle with a complex innervation, and (3) several elastic strands with numerous nerve fibres ending on them. In the Brachyura the organs under (1) seem to be missing in the thoracico-coxal articulation, while the muscular receptor has a more complicated structure. Both in the Brachyura and the Astacura different species show variations in the detailed arrangements of the receptors.

Several of these organs show an unusual feature in that the cell body of the receptor neuron is not situated near its peripheric endings. It is understandable that doubts may arise as to whether the observations were correct as to this point. As a rule, when the search for some nervous element has been unsuccessful, it is advisable, instead of stating that they are missing, to say that they have not been found, and, admittedly, the observations recorded in the present paper may be liable to amendments in the future. In this case, however, the histological evidence can be interpreted in only one sense, which is that the fibres terminating on the tendinous parts of the muscular receptors and on the innervated elastic strands are emitted by neurons of which the cell bodies are situated in the central nervous system. As these fibres are obviously afferent in nature the conclusion must be drawn that in the Crustacea sensory neurons can develop, as in vertebrates, a long process which behaves functionally as a dendrite, but which otherwise may be indistinguishable from an axon. In the organs under consideration various stages in the elongation of the dendrites can be observed. In the muscular receptors in *Homarus* the fibres of the sensory neurons differ in appearance from ordinary nerves; as they do not extend far from the ganglion there is not much difference in shape and dimensions between them and those of the receptor organs in the dorsal muscles. In the 8th thoracic segment of *Homarus*, where the receptor organs originate at a greater distance from the neural cord, the dendrites of their sensory neurons are elongated and look like ordinary nerve fibres. The same is true of their aspect in crabs, and in the hinder thoracic segments in *Astacus*. The nerves of the innervated elastic strands have various dispositions, some, such as those of the additional strands in *Homarus* and of the depressor receptor in *Carcinus*, associate with the nerve fibres of the muscular receptor, while others travel in the nerve trunks of the leg, and, until their branches are seen to reach the strands, are practically indistinguishable among the ordinary fibres.

The structure of the muscular receptor in *Homarus* resembles in certain features that of the receptor organs in the dorsal muscles of the same animal. The aspect of the muscle and the pattern of its motor innervation appear almost identical. The connexion of the muscle and the receptor neurons is effected on the same principle, that is, the dendrites of the latter end on the connective tissue linked with the muscle but not on the muscular tissue itself. On the other hand there are important differences. One, the absence of the peripheral nerve-cell bodies has just been discussed; the other is in the position of the sensory terminations, which is at the end of the receptor muscle in the leg, but always at some distance from the attachments of the receptors in the dorsal muscles. A further difference is the presence in the coxal receptors of two sensory neurons (or even three, as in *Maia*) ending at the same muscle. The question arises whether there is evidence for considering them to have various functions. Their aspect in *Homarus* does not provide support for such a view. In crabs, however, they exhibit such differentiation in the disposition of their endings that the assumption of their having different natures has a high degree of probability.

The elastic receptor of the thoracico-coxal joint in *Homarus* and *Astacus* is so like that of the coxo-basipodite joint and the other receptors farther down the leg, that it must be considered as belonging to the same series. It is strange that it should be well developed and situated so near the muscular receptor in these animals and yet be, apparently, absent in the other species investigated.

As regards the innervated elastic strands, their very existence, in the vicinity of other receptor organs, is puzzling, apart from their structure. The ending of thick nerve fibres in elastic strands is a most unusual feature. Moreover, the levator and depressor systems receive different sets of nerves, of various kinds. It is difficult to assess how much value may be attached to these differences, but it is reasonable to assume that each anatomical difference is likely to have a functional meaning and sets a problem to be solved.

The suspensory ligament has been described as a component of the system of innervated strands and it certainly plays an important role in it. It is, however, not evident whether this is its only function. Being made of connective tissue it can only serve to offer resistance to some pulling force, but why this should be necessary in this region, apart from giving a point of attachment to the innervated strands, it is difficult to say. To the conjecture that it might give support to the ganglion it may be objected that its firm attachments are at the endosternites lateral to the median line of the body and, besides, in the 8th thoracic segment where the ligament is particularly well developed, it cannot have such a function because of the more anterior position of the ganglion.

As stated in the introductory remarks, the occurrence of proprioceptors in the coxal articulations was predicted. The possibility of their existence was adumbrated by Bethe (1897) who, experimenting on compensatory eye movements in *Carcinus maenas*, expressed the view that besides the reflexes elicited by the eyes and the statocysts, a 'third factor' may play a role. Dijkgraaf (1956*a*, *b*) has demonstrated that proprioceptors must be there. They are indeed. But now the question of their function remains to be answered, for it seems most unlikely that such organs have no other role than to participate in the compensatory eye reflexes in which, as Bethe's and Dijkgraaf's experiments have shown, they play only a subordinate part.

Their number, variety and structure indicate that there is in the coxal region of decapod crustaceans a complex apparatus whose elements, or a part of them, are instrumental in registering the position of the legs with respect to the thorax. What may be the function of each of them remains to be determined.

SUMMARY

In the 4th to 8th thoracic segments of decapod Crustacea, at the bases of the peraeopods, sensory organs of various kinds have been found. They have been observed in *Homarus vulgaris*, *Astacus astacus*, *Palinurus vulgaris*, *Eupagurus bernhardus*, *Carcinus maenas*, *Maia squinado* and described in greater detail for *Homarus*, *Carcinus* and *Maia*.

In all species examined there is in each peraeopod a proprioceptor consisting of a thin muscle with various nerve elements which extends from a point of the endophragmal skeleton situated near the ventral ganglia to the anteroventral part of the coxa. In the 4th to 7th thoracic segments of *Homarus*, in the 4th to 8th of *Palinurus* and *Astacus*, and in the 4th of the Brachyura, the receptor muscle is proximally attached to the tip of a chitinous projection of the endophragmal skeleton for which the term 'receptor rod' is proposed. In other instances it originates on the plate of the endosternite.

The nerve components of this 'muscular receptor' are of two sorts: (1) motor nerves spreading their branches over the whole length of the muscle, and (2) processes of the sensory neurons distributing their ramifications on the connective tissue associated with the muscle at its proximal end. The arrangement of the sensory elements is different in various species, but they all exhibit one feature, unusual in invertebrates, viz. their cell-bodies are situated within the central nervous system and not outside it.

In *Homarus* and *Astacus* there is a second receptor organ having the same attachments as the former and running close to it. It has been called 'elastic receptor' since it is composed of fibres with elastic properties; they form a sort of tube in which a row of bipolar nerve cells and their processes are enclosed. In all other species investigated this organ appears to be missing.

In all species investigated there is a sensory organ spanning the articulation between the coxa and the basipodite. It is made up of a strand of elastic

fibres and a row of sensory nerve cells. This coxo-basipodite receptor belongs to a system of receptors of similar structure which occur in all articulations of the legs.

A different system of sensory organs consists of innervated elastic strands. As they are associated with the two muscles inserting into the basipodite they may be called the 'depressor receptor' and 'levator receptor' respectively. In *Homarus* and *Astacus* this system consists of two strands arising on the receptor rods and two others connected with a special connective tissue band termed the suspensory ligament. In *Carcinus* and *Cancer* it is simpler, being represented by two strands originating near the proximal end of the receptor muscle. In *Maia* these organs have not been found. The strands are abundantly supplied with nerve fibres considered to be processes of sensory nerve cells located in the central nervous system.

REFERENCES

ALEXANDROWICZ, J. S., 1951. Muscle receptor organs in the abdomen of *Homarus vulgaris* and *Palinurus vulgaris*. *Quart. J. micr. Sci.*, Vol. 92, pp. 163–99.

BALSS, H., 1941. Decapoda. *Bronn's Tierreich*, Lief 2.

BETHE, A., 1897. Das Nervensystem von *Carcinus maenas.* I.T. I.Mitt. *Arch. mikr. Anat.*, Bd. 50, pp. 460–546.

COCHRAN, D. M., 1935. The skeletal musculature of the blue crab, *Callinectes sapidus* Rathbun. *Smithsonian misc. Coll.*, Vol. 92, No. 9, pp. 1–76.

DIJKGRAAF, S., 1956*a*. Kompensatorische Augenstieldrehungen und ihre Auslösung bei der Languste (*Palinurus vulgaris*). *Z. vergl. Physiol.*, Bd. 38, pp. 491–520.

—— 1956*b*. Ueber die kompensatorischen Augenstielbewegungen bei Brachyuren. *Pubbl. Staz. zool. Napoli*, Vol. 28, pp. 341–58.

DRACH, P., 1939. Mue et cycle d'intermue chez les Crustacés Décapodes. *Ann. Inst. Océanogr. Monaco*, T. 19, pp. 103–391.

MILNE EDWARDS, H., 1834. *Histoire naturelle des Crustacés.*

—— 1851. Observations sur le squelette tégumentaire des Crustacés Décapodes. *Ann. Sci. nat. (zool.)*, T. 16, pp. 222–91.

PEARSON, J., 1908. *Cancer. L.M.B.C. Memoirs*, No. 16.

SCHMIDT, W., 1915. Die Muskulatur von *Astacus fluviatilis* (*Potamobius astacus* L.). *Z. wiss. Zool.*, Bd. 113, pp. 165–251.

EXPLANATION OF PLATES

PLATE I

All photomicrographs are made from *Homarus vulgaris.*

Fig. 1. Receptor organs of the thoracico-coxal articulation of the 5th thoracic segment in connexion with the ganglion seen from above. On the left side both the muscular and the elastic receptors are present; on the right side the elastic receptor is removed. *rec.r.*, upper parts of the receptor rods cut from the endosternites.

Fig. 2. Proximal parts of the muscular (*m.rec.*) and the elastic (*el.rec.*) receptors of the right side.

Fig. 3. Muscular receptor of the right side from Fig. 1 under higher magnification.

Fig. 4. Proximal part of the muscular receptor of the left side. Note its attachment to the receptor rod (*rec.r.*). In the bundle of nerves connecting the receptor with the ganglion are included fibres of the additional strands (cf. Text-figs. 3, 5); the strands themselves are not seen.

Fig. 5. Proximal part of the additional strand of the levator receptor with nerve fibre branching in it.

Fig. 6. Middle part of the coxo-basipodite receptor. The axons of nerve cells are in this preparation more apart than usual; in their proximal course they unite into a compact bundle.

PLATE II

Fig. 7. *Homarus vulgaris*. Suspensory ligament (*lig.*) of the right side of the 2nd thoracic segment with the main strands of the levator (*lev.rec.*) and depressor (*depr.rec.*) receptors. Additional strands are removed.

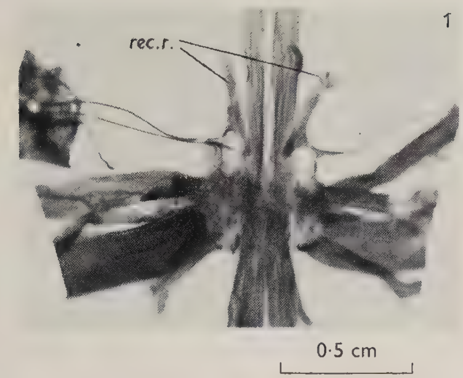
Fig. 8. *H. vulgaris*. Nerve fibres of the depressor receptor (cf. Text-fig. 5). *ad.*, branches of the fibre of the additional strand; the division of the nerve fibre at a distance from the main strand, as seen in this preparation, is less common.

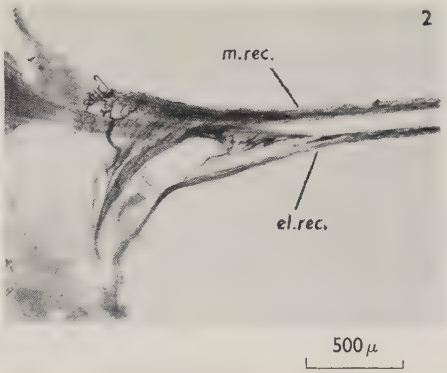
Fig. 9. *Carcinus maenas*. Receptor rod with the muscular receptor (*m.rec.*). The nerves are incomplete and somewhat displaced.

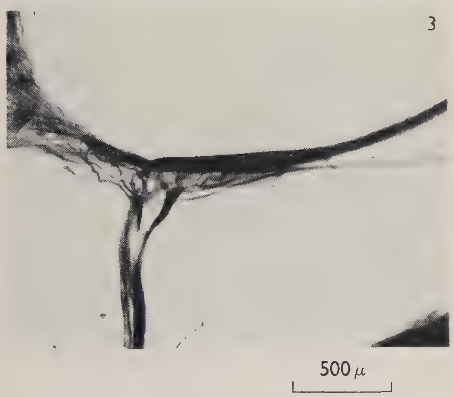
Fig. 10. *Maia squinado*. Receptor rod with the muscular receptor (*m.rec.*) and its nerves. *a*, fibre ending on the tendon; *b*, two fibres branching before reaching the receptor muscle; in the photograph their outlines are confluent but in fact there are two.

Fig. 11. *Carcinus maenas*. Proximal part of the muscular receptor of the 8th thoracic segment of the left side. *depr.rec.*, depressor receptor; *lev.rec.*, levator receptor. Note the nerve fibres of the levator receptor (cf. Text-fig. 8).

Fig. 12. *Maia squinado*. Proximal part of the muscular receptor of the 6th segment of the left side with branches of the nerve fibre innervating the tendon and with the two nerve fibres penetrating into the strand flanking the receptor muscle.


1
rec.r.
0·5 cm


2
m.rec.
el.rec.
500 μ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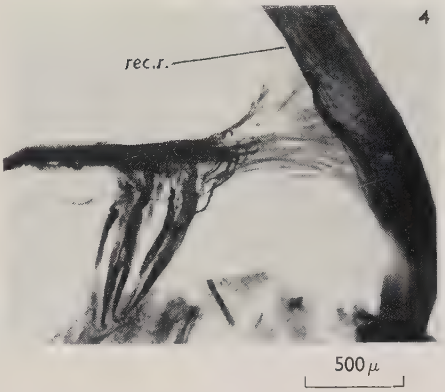
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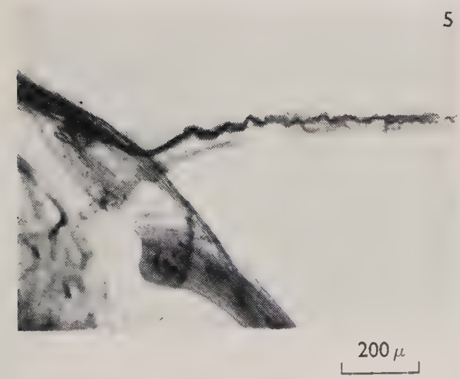
rec.r.
500 μ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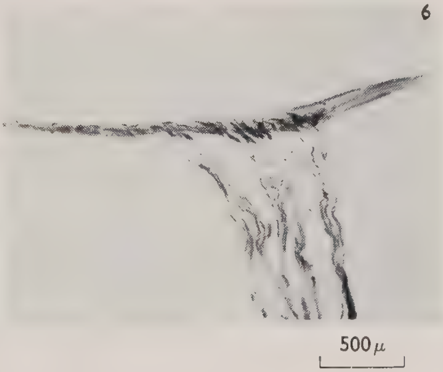
6
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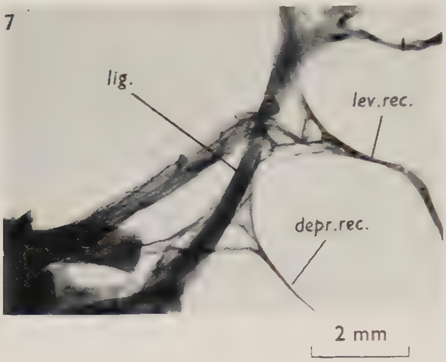
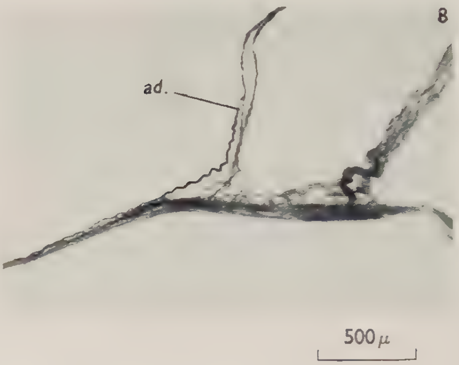
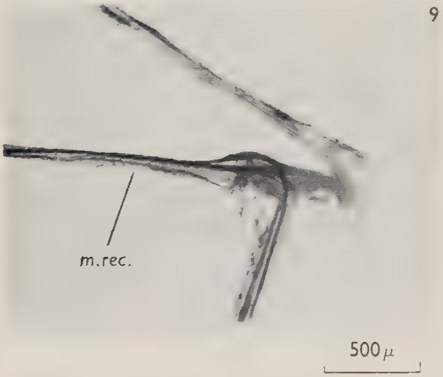
7


lig.
lev.rec.
depr.rec.
2 mm

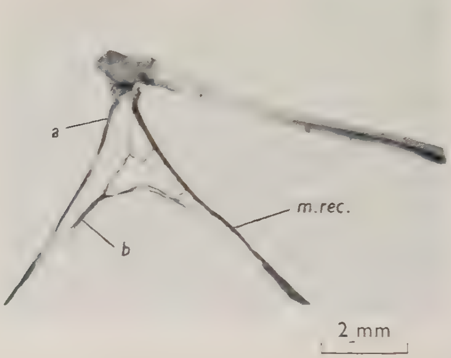
8


ad.
500 μ

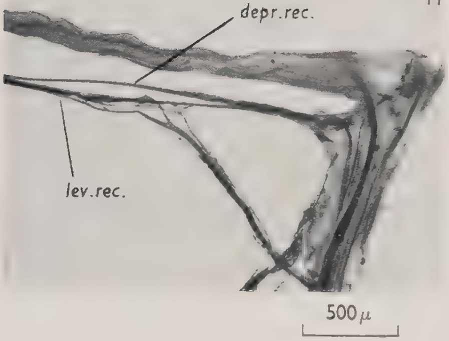
9


m.rec.
500 μ

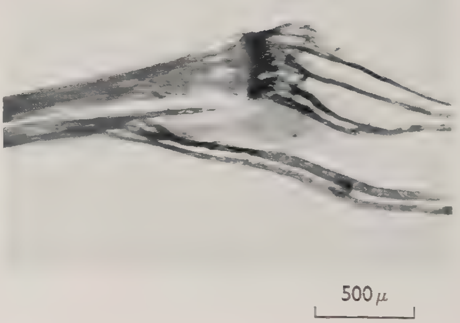
10


a
m.rec.
b
2 mm

11


depr.rec.
lev.rec.
500 μ

12


500 μ

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SPECTRAL COMPOSITION OF THE LIGHT OF *CHAETOPTERUS*

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(Text-figs. 1–13)

The light of *Chaetopterus* has a bluish hue. From visual examination Lankester (1868) judged that the emission spectrum extended from about 440 to 550 mμ (wavelengths estimated by Harvey, 1952). Other polychaetes said to produce bluish luminescence are found in the families Alciopidae, Syllidae and Terebellidae. The light of Tomopteridae and Cirratulidae is described as yellow or yellow-green (Harvey, 1955). The spectral composition of the light of polynoids has now been measured. It is yellow-green in colour, with maximal emission at about 515 mμ (Nicol, 1957).

The spectral composition of the light of *Chaetopterus variopedatus* is here described. Various physiological aspects of the response, some of them pertinent to the present investigation, have already been dealt with (Nicol, 1952*a–c*). The light is produced by a luminescent secretion discharged into the surrounding sea water. When the glands on segment XII discharge, the light rises to maximal intensity in about 14 sec, and the whole response lasts some 5 min.

MATERIALS AND METHODS

Chaetopterus was removed from its tube, and the anterior region (head) was cut off. The head was pinned out in a black dish, and light was evoked by electrical stimulation of the luminescent glands lying at the bases of the aliform notopodia (segment XII) (Nicol, 1952*a*, *b*).

To analyse the spectral composition of the light, a multiplier phototube and a series of coloured filters were used. The photomultiplier was connected to a cathode-ray oscilloscope, and photographic records were made of the deflexion of the oscilloscope trace on moving paper.

The filters used were Ilford spectrum filters nos. 601–608, covering the visible range (about 400–700 mμ), Chance's purple (OV 1), and an ultra-violet filter transmitting from about 300–400 mμ. A set of filters was mounted in an opaque disc which could be rotated beneath the cathode of a photomultiplier tube. Apertures were cut at regular intervals about the margin of the disc, and a filter was placed over each aperture. The disc was arranged so that the filter-covered apertures passed across the face of the photocathode (arrangement shown in Fig. 1). The dish containing the animal was placed under the disc, close to the filters.

Since the light varies in intensity, it was necessary to have some method of registering changes in light intensity, while records of intensity of filtered light in different spectral regions were obtained. This was accomplished by using a series of identical filters (hereafter called reference filters), one of which was placed between each of the other spectral filters. By this means it was possible to gauge the relative light intensity at the beginning and end of each measurement made with a given spectral filter. For reference filters I employed either Ilford blue-green 603, or Ilford green 604. From the intensity of the light passed by the reference filters, it was possible to correct the records obtained with other spectral filters, so that all the data referred to light of the same initial energy-content before filtration.

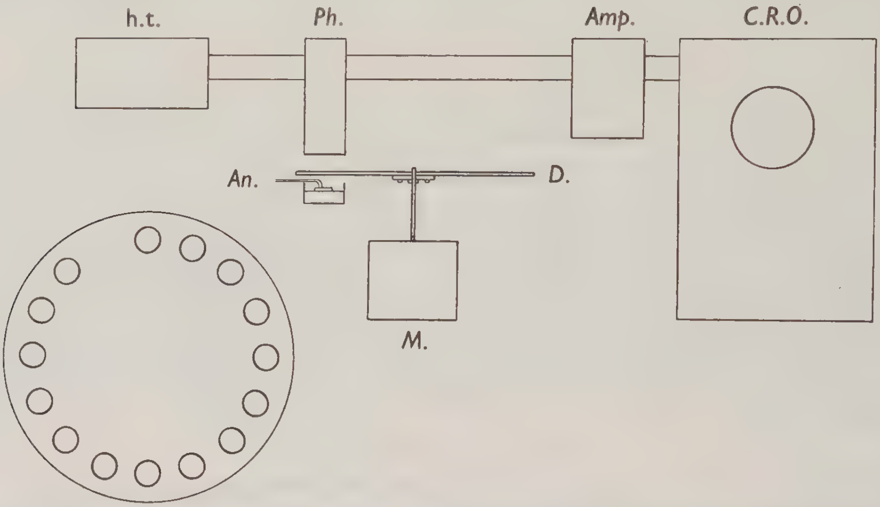


Fig. 1. Diagram of apparatus. *Amp.*, amplifier; *An.*, animal; *D.*, disc; *C.R.O.*, cathode-ray oscilloscope; *h.t.*, high tension supply; *M.*, variable speed motor. The disc is shown in surface view at the lower left.

In order to reduce the disparity in magnitude of responses given with the various filters, neutral filters were used in conjunction with Ilford spectrum filters nos. 601–604. They were either Chance's neutral glass or Ilford neutral density filters. Four combinations of filters that were used are shown in Table 1. The filters are given in the order in which they passed across the face of the multiplier phototube. A blank opaque space was left between each pair of contiguous filters to separate the records clearly. At one point on the disc there was a double blank space as a position-marker, to show when the disc had made one complete rotation, and to provide a means of relating the seriated responses to the filters in their order of rotation. Thus, in disc I, when the filters were rotated in order from red to violet, the photographic trace would show: nil deflexion, long duration (= double space); deflexion (= blue-green filter); nil deflexion, short duration (= single space); deflexion (= red filter);

nil deflexion, short duration (=single space); deflexion (=blue-green filter); etc. Some records, described later, are shown in Figs. 2 and 3.

The transmission of all filters was measured in a spectrophotometer (Unicam, S.P. 500).

The photomultiplier was an E.M.I. no. 6685, having high sensitivity in the violet and very low sensitivity in the red. The spectral sensitivity of the

TABLE 1. FOUR COMBINATIONS OF FILTERS, LISTED IN ORDER OF ROTATION

Disc I

Double space

1. Ilford blue-green 603 + Ilford neutral density (D. 0·5)
2. Ilford red 608
3. Ilford blue-green 603 + Ilford neutral density (D. 0·5)
4. Ilford orange 607
5. Ilford blue-green 603 + Ilford neutral density (D. 0·5)
6. Ilford yellow 606
7. Ilford blue-green 603 + Ilford neutral density (D. 0·5)
8. Ilford yellow-green 605
9. Ilford blue-green 603 + Ilford neutral density (D. 0·5)
10. Ilford green 604 + Ilford neutral density (D. 0·5)
11. Ilford blue-green 603 + Ilford neutral density (D. 0·5)
12. Ilford blue 602 + Ilford neutral density (D. 0·5)
13. Ilford blue-green 603 + Ilford neutral density (D. 0·5)
14. Ilford violet 601
15. Ilford blue-green 603 + Ilford neutral density (D. 0·5)

Disc II

Double space

1. Ilford green 604
2. Ilford orange 607
3. Ilford green 604
4. Ilford yellow 606
5. Ilford green 604
6. Ilford yellow-green 605
7. Ilford green 604
8. Ilford blue-green 603 + Chance neutral ON 31
9. Ilford green 604
10. Ilford blue 602 + Chance neutral ON 32
11. Ilford green 604
12. Ilford violet 601 + Chance neutral ON 33
13. Ilford green 604

Disc III

Double space

1. Ilford green 604
2. Ultra-violet
3. Ilford green 604
4. Ilford yellow 606
5. Ilford green 604
6. Ilford yellow-green 605
7. Ilford green 604
8. Ilford blue-green 603 + Chance neutral ON 31
9. Ilford green 604
10. Ilford blue 602 + Chance neutral ON 32
11. Ilford green 604
12. Ilford violet 601 + Chance neutrals ON 32 + ON 33
13. Ilford green 604
14. Chance OV 1
15. Ilford green 604

Disc IV

Double space

1. Ilford red 608
2. Ilford green 604
3. Ilford orange 607
4. Ilford green 604
5. Ilford yellow 606
6. Ilford green 604
7. Ilford yellow-green 605
8. Ilford green 604
9. Ilford blue-green 603 + Chance neutral ON 31
10. Ilford green 604
11. Ilford blue 602 + Chance neutral ON 32
12. Ilford green 604
13. Ilford violet 601 + Chance neutral ON 33
14. Ilford green 604
15. Chance OV 1
16. Ilford green 604

photocathode of this tube was determined by the National Physical Laboratory. Voltage for the photomultiplier was supplied by a stabilized power pack. The mains voltage for oscilloscope and power pack was held steady by a voltage stabilizer.

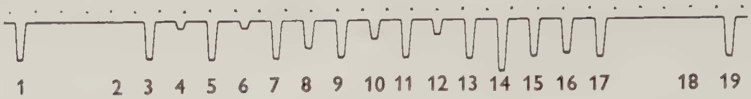


Fig. 2

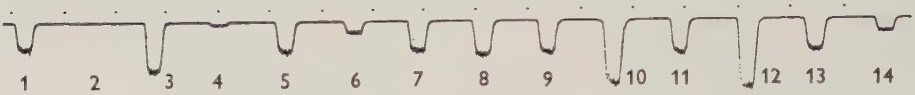


Fig. 3

Fig. 2. Photographic record of oscillograph deflexions given by artificial light source (lamp 2360° K + Chance blue-green OB 2), interrupted by rotation of disc IV. Responses as follows: 1, green 604; double space; 2, red 608; 3, green 604; 4, orange 607; 5, green 604; 6, yellow 606; 7, green 604; 8, yellow-green 605; 9, green 604; 10, blue-green 603; 11, green 604; 12, blue 602; 13, green 604; 14, violet 601; 15, green 604; 16, purple OV 1; 17, green 604; double space; 18, red 608; 19, green 604.

Fig. 3. Similar record for the light of *Chaetopterus*, interrupted by disc III. Responses as follows: 1, green 604; 2, ultra-violet; 3, green 604; 4, yellow 606; 5, green 604; 6, yellow-green 605; 7, green 604; 8, blue-green 603; 9, green 604; 10, blue 602; 11, green 604; 12, violet 601; 13, green 604; 14, deep purple OV 1. Time, both records, $\frac{1}{5}$ sec.

In order to calculate the relative spectral energy of a light source by the method here adopted, it is necessary to know the combined effect of spectral sensitivity of the photomultiplier (S_λ) and spectral transmission of each filter (T_λ). Let R_x be the response for a given filter x, and E_λ the relative spectral energy of emitted light. Then

$$R_x \propto E_\lambda \int S_\lambda T_\lambda \, d\lambda,$$

$$\therefore \; E_\lambda \propto \frac{R_x}{\int S_\lambda T_\lambda \, d\lambda}.$$

Plots of $S_\lambda T_\lambda$ against λ are given in Figs. 4 and 5. The areas under curves $S_\lambda T_\lambda$ were used to represent the integrals for each filter

$$\eta_x = \int S_\lambda T_\lambda \, d\lambda.$$

The mean wavelength (mean λ) for each filter was taken from the centre of gravity of the areas under the curves. Values for η_x and mean λ are given in Table 2.

All measurements of *Chaetopterus* light were made at a room-temperature of 18–19° C.

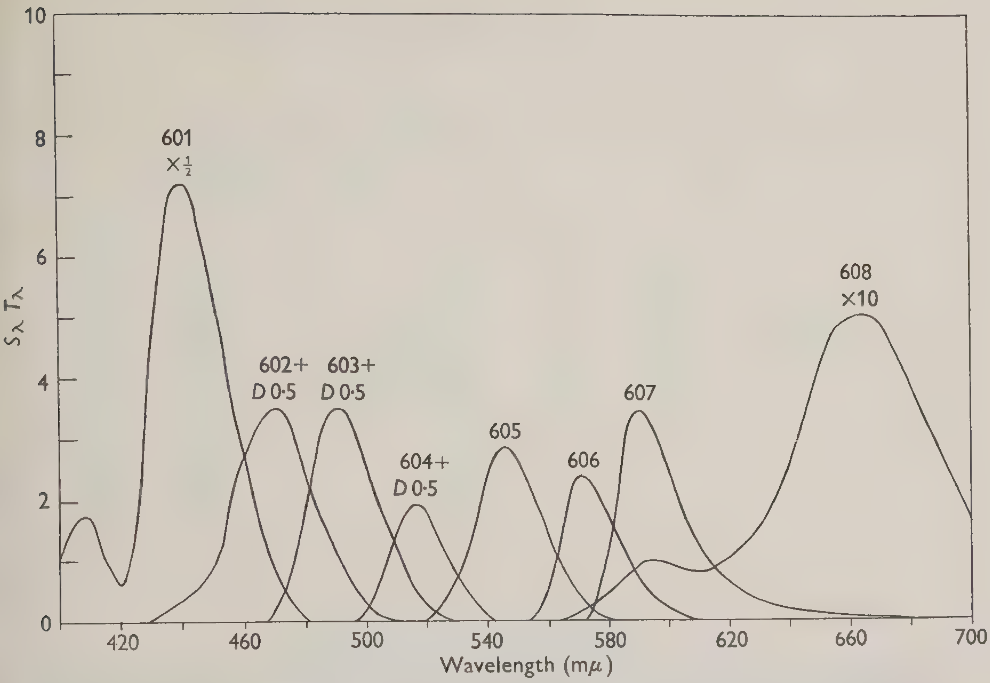


Fig. 4. Plots of $S_\lambda T_\lambda$ against λ for the series of Ilford gelatine filters used in disc I. S_λ = sensitivity of photomultiplier 6685; T_λ = transmission of filters. An Ilford neutral density filter (density D. = 0·5) was used with each of filters 602 to 604. All filters were mounted between two pieces of Perspex.

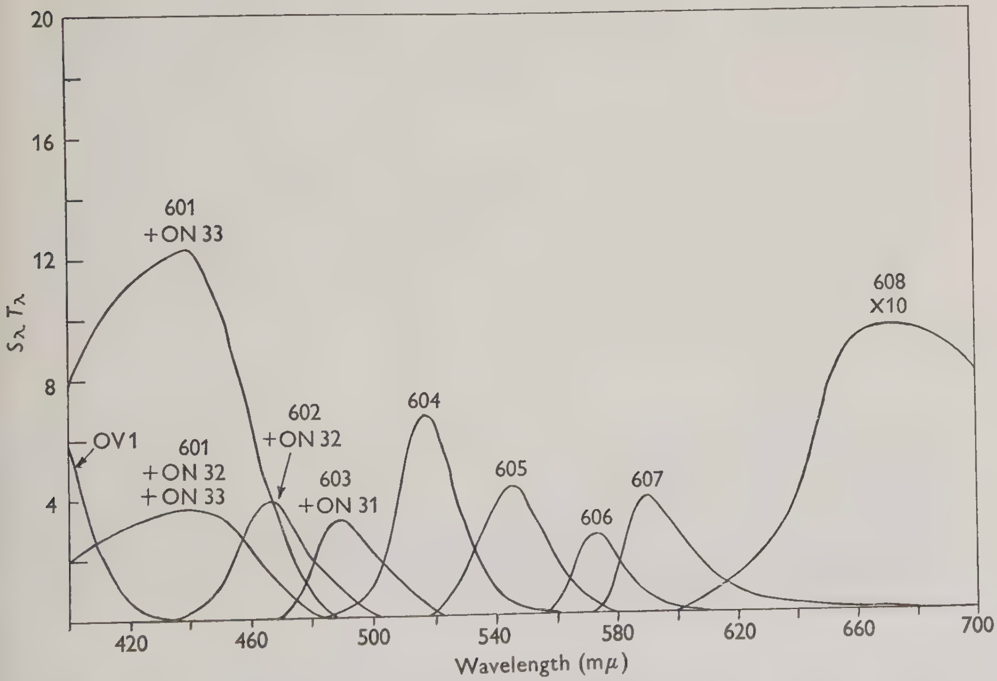


Fig. 5. Plots of $S_\lambda T_\lambda$ for the various filters used in discs II, III and IV. Filters 601 to 608 were gelatine, mounted in glass; the remainder glass.

TABLE 2. VALUES FOR INTEGRALS $\eta_\lambda = \int S_\lambda T_\lambda d\lambda$ (COMBINED SENSITIVITY OF PHOTOMULTIPLIER AND TRANSMISSION OF FILTERS)

Disc I			Discs II, III and IV		
Filter	η_x	Mean λ (mμ)	Filter	η_x	Mean λ (mμ)
601	95·23	439	Ultra-violet	616	*ca.* 370
602 + neutral D. 0·5	22·60	467	Chance OV 1	700·60	383
603 + neutral D. 0·5	19·14	490		119·60	403*
604 + neutral D. 0·5	8·23	514	601 + ON 33	138·90	434
605	15·33	545	601 + ON 32 + ON 33	42·33	432
606	10·87	572	602 + ON 32	22·75	465
607	19·79	595	603 + ON 31	17·30	490
608	6·02	658	604	29·02	514
			605	24·13	548
			606	10·92	573
			607	23·89	595
			608	5·53	661

* Visible range.

OBSERVATIONS

ESTIMATION OF ACCURACY OF THE METHOD

In order to test the accuracy of the method, filtered light of known spectral composition was measured by means of a disc of filters, photomultiplier and oscilloscope. Constant light was provided from a substandard lamp of colour temperature 2360° K, obtained from the National Physical Laboratory. A blue filter (Chance's blue-green OB 2) was placed between the lamp and apparatus. Disc IV was used, covering the spectrum from the ultra-violet (*ca.* 350 mμ) to the infra red (above 700 mμ) (Table 1). The disc was rotated at slow speeds, around 0·5–0·25 rev./min. Photomultiplier no. 6685, it will be noted, has very low sensitivity in the red. Curves for relative spectral energy of the lamp and for transmission of the filter OB 2 are given in Fig. 6. In the same figure is shown the calculated spectral composition of the light that passed through the filter and that was being measured (curve 3). Noise level was reduced by a condenser (0·05 μF) placed across the input of the oscilloscope.

The responses (R_x) obtained with the several filters are given in Table 3, together with values for relative spectral energy,

$$Q_x = \frac{R_x}{\eta_x}.$$

The values for Q_x (relative spectral energy) have been plotted on Fig. 6 against mean λ (from curves $S_\lambda T_\lambda$).

Owing to the fact that wavelengths towards maximal spectral emission contribute more effectively than others towards the response with each filter, it is desirable to make an appropriate correction to the measurements. This was carried out as follows.

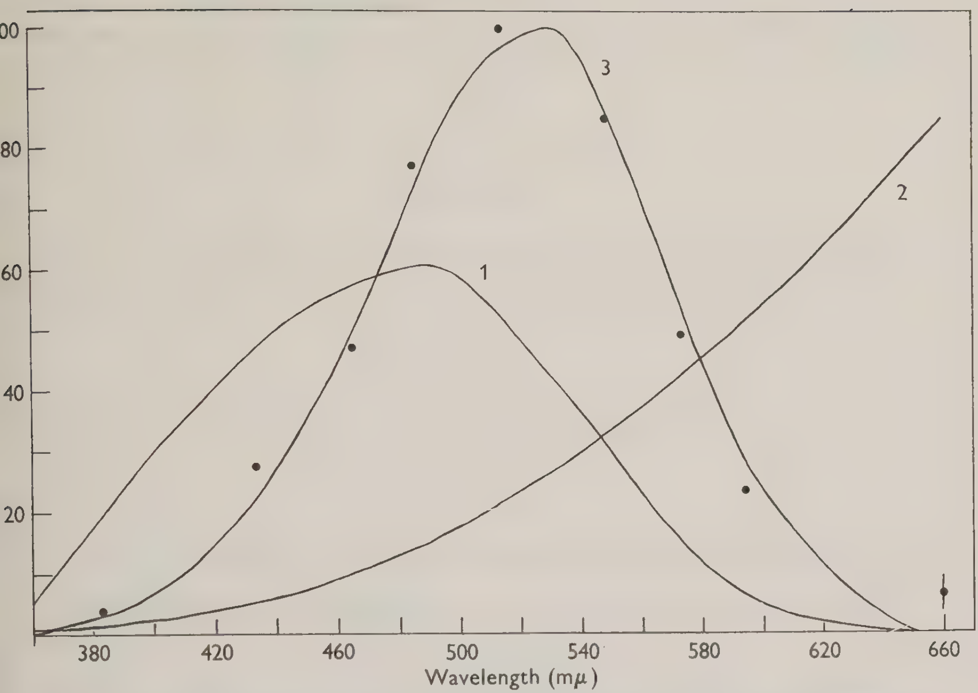


Fig. 6. Curves for (1) transmission (%) of a blue filter, Chance OB 2; (2) spectral emission of a substandard lamp (colour temperature 2360° K), and (3) calculated spectral composition of light passed by filter OB 2 ($T_\lambda \mathcal{J}_\lambda$). Relative $\mathcal{J}_\lambda$ based on $\mathcal{J}=1$ at $\lambda=590$ mμ (from Skogland, 1929). Relative $T_\lambda \mathcal{J}_\lambda$ based on $T\mathcal{J}=100$ at $\lambda=530$ mμ. Curve (2), × 50. The points are measured values for spectral composition of light passed by filter OB 2.

TABLE 3. MEASUREMENT OF SPECTRAL COMPOSITION OF AN ARTIFICIAL LIGHT SOURCE

Lamp of colour temperature 2360° K + Chance blue-green filter OB 2. Responses and first approximation of relative spectral energy.

Filter	Mean λ (mμ)	Response R_x	$R_x/\eta_x=Q_x$
OV 1	383	10	0·0143
601 + ON 33	434	15	0·1080
602 + ON 32	465	4·2	0·1846
603 + ON 31	490	5·2	0·3006
604	514	11·3	0·3894
605	548	8·0	0·3315
606	573	2·1	0·1923
607	595	2·2	0·0921
608	661	0·15?	0·0271?

From the points for Q_x in Fig. 6, a first approximate curve for relative spectral emission was drawn. Let the values on this curve be $E_{\lambda A}$. Then the ratios

$$\rho_1 = \frac{\int E_{\lambda A} S_\lambda T_{\lambda x}}{\int E_{\lambda A} S_\lambda T_{\lambda 604}}$$

were calculated, and used to predict the experimental results.

The responses R_x actually obtained with each filter give the true ratios

$$\rho_2 = \frac{R_x}{R_{604}}.$$

When ρ_1 and ρ_2 differ, the latter is used to correct the experimental results, viz.

$$\frac{\rho_2}{\rho_1} = \rho_3.$$

$Q_x \rho_3$ = corrected relative spectral energy for each filter, when $Q_{604} = 100$.

TABLE 4. SPECTRAL COMPOSITION OF ARTIFICIAL LIGHT SOURCE

(correction of first approximate results)

Filter	$\int E_\lambda S_\lambda T_\lambda(\zeta_x)$	$\zeta_x/\zeta_{604} = \rho_1$	$R_x/R_{604} = \rho_2$	$\rho_2/\rho_1 = \rho_3$	$Q_x\rho_3$ $\lambda_{530} \equiv 100$	Mean λ
OV 1	201·0	1·16	0·885	0·763	2·79	395
601 + ON 33	227·7	1·32	1·33	1·007	27·83	445
602 + ON 32	66·66	0·385	0·372	0·966	45·63	466
603 + ON 31	79·34	0·459	0·460	1·002	77·08	491
604	173·0	—	—	—	97·56	516
605	108·75	0·629	0·708	1·126	95·51	542
606	26·67	0·154	0·186	1·208	59·45	572
607	31·00	0·179	0·195	1·089	25·65	592
608	2·27	0·013	0·013?	1?	6·93?	622

In Table 4 are given the details of these calculations for filtered blue light from the substandard lamp. The second column lists areas of the curves $E_{\lambda A} S_\lambda T_{\lambda x}$ plotted against $\lambda(\zeta_x)$. Columns 3–5 give values of ρ_1, ρ_2 and ρ_3, for the various filters. The sixth column $Q_x\rho_3$ gives relative spectral energy for each filter, based on $\lambda_{530} \equiv 100$, the estimated region of maximal emission. The last column shows recalculated values for mean λ, based on curves $E_{\lambda A} S_\lambda T_{\lambda x}$.

The final corrected values for relative spectral energy for each filter are plotted on Fig. 7, together with the calculated relative energy curve (based on $\mathcal{J}_\lambda T_\lambda$). The degree of agreement, which can be seen from inspection, seems reasonable enough to trust the method for measurement of animal luminescence.

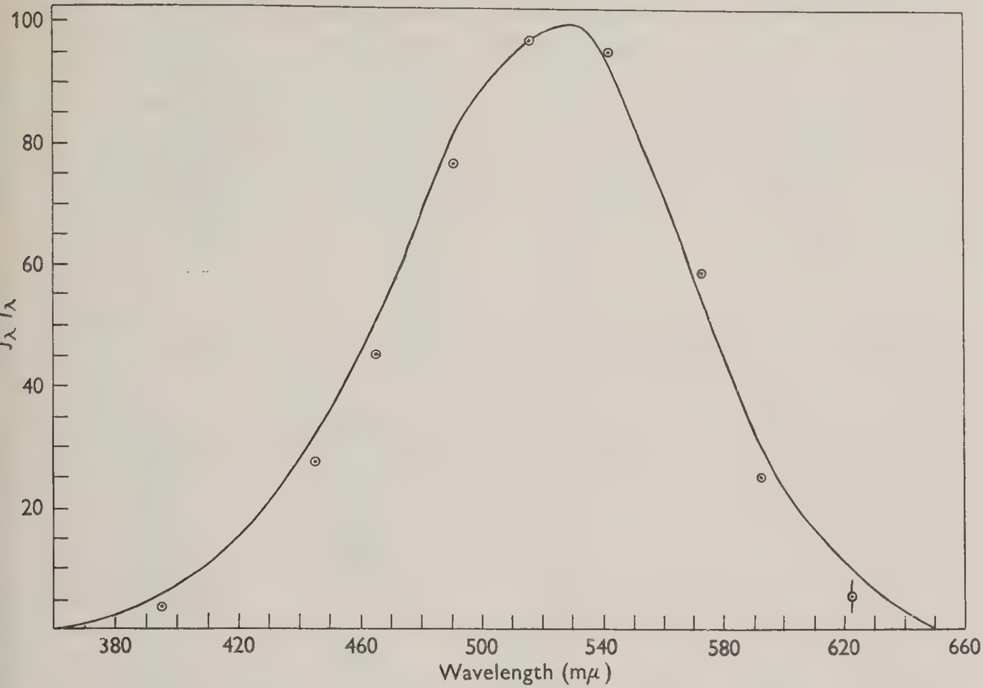


Fig. 7. Curve for relative spectral energy of artificial light (lamp 2360° K + Chance OB2). The points are corrected measurements made with the various filters, and plotted against mean λ (determined from curves $E_{\lambda A}S_\lambda T_{\lambda x}$).

MEASUREMENTS OF THE LIGHT OF *CHAETOPTERUS*

As a first attempt, the light of *Chaetopterus* was measured by means of disc I, containing Ilford filters 601 to 608. This disc was designed for green light. It was rotated at various speeds, varying from 1 to 3 rev./sec. The data obtained are summarized in Table 5, and first approximate estimations of relative spectral energy are plotted against λ in Fig. 8 (mean λ for each filter based on curves $S_\lambda T_\lambda$).

From these preliminary results it appeared that the light possessed a maximum well into the blue, around 460 mμ, and discs II and III were devised, accordingly, to extend the spectral range of analysis. Discs II and III were spun at low speeds, around 0·5 rev./min, at which rate it was possible to use a condenser (0·05 μF) to filter off most of the noise. The data obtained with discs II and III are shown in Table 6, and values for relative spectral energy are plotted in Fig. 9 against mean λ (derived from curves $S_\lambda T_\lambda$).

In order to correct these values, the same procedure was employed, as described in the previous section. First approximate values for relative

TABLE 5. SPECTRAL COMPOSITION OF THE LIGHT OF *CHAETOPTERUS*

(Results obtained with disc I)

Filter	Mean λ (mμ)	Response R_x	$R_x/\eta_x = Q_x$
601	439	5·8749	0·0617
602 + D. 0·5	467	1·9872	0·0879
603 + D. 0·5	490	1·000	0·0522
604 + D. 0·5	514	0·2959	0·0360
605	545	0·2098	0·0137
606	572	0·0566	0·0052
607	595	0·0664	0·0034
608	658	—	—

TABLE 6. SPECTRAL COMPOSITION OF THE LIGHT OF *CHAETOPTERUS*

(Results obtained with discs II and III)

Filter	Mean λ (mμ)	Response R_x	$R_x/\eta_x = Q_x$
Ultra-violet	*ca.* 370	0	0
OV 1	403	0·365	0·003052
601 + ON 33	434	6·0029	0·043405
601 + ON 32 + ON 33	432	2·445	0·057761
602 + ON 32	465	1·9283 1·9233	0·08465 mean
603 + ON 31	490	0·9982	0·057699
604	514	1	0·034459
605	548	0·3902	0·016171
606	573	0·0913	0·008361
607	595	0·0686	0·002871
608	661	0	0

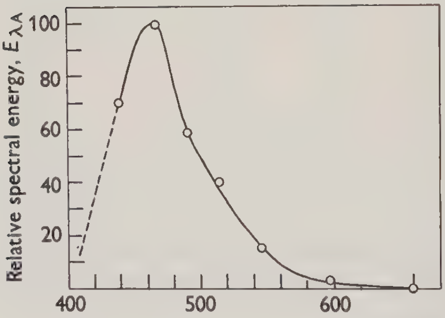


Fig. 8

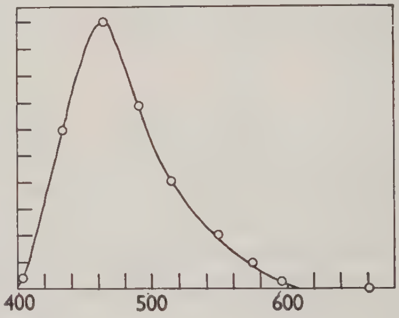


Fig. 9

Fig. 8. Spectral composition of the light of *Chaetopterus*. Approximate curve based on records obtained with disc I. Mean λ for each filter obtained from curves $S_\lambda T_\lambda$.

Fig. 9. Spectral composition of the light of *Chaetopterus*. Approximate curve based on records obtained with discs II and III. Mean λ for each filter obtained from curves $S_\lambda T_\lambda$.

spectral emission $E_{\lambda A}$ were estimated from a curve resembling that in Fig. 9, and these values were used to draw curves for $E_{\lambda A}S_{\lambda}T_{\lambda x}$ (Fig. 10). Computed values are listed in Table 7, together with subsequent calculations to determine corrected values for spectral emission. Final values are collected in Fig. 11, which depicts a corrected spectral emission curve. Maximal emission occurs at about 465 mμ, well into the blue. There is no emission in the ultra-violet, and emission in the red, above 600 mμ, is negligible.

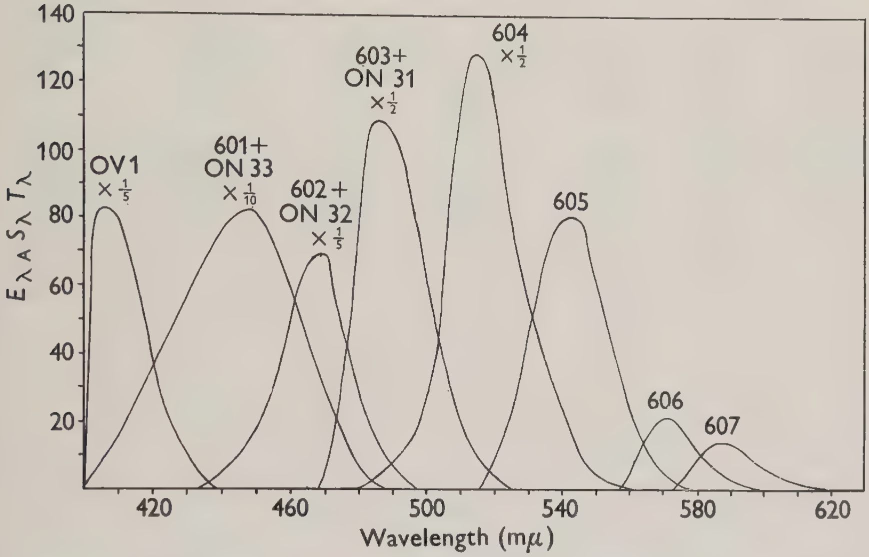


Fig. 10. Curves for $E_{\lambda A}S_{\lambda}T_{\lambda}$ against λ, based on first approximate measurements of relative spectral emission of *Chaetopterus* light.

COMMENT

Most of the light of *Chaetopterus* is concentrated in the blue region of the spectrum, more so than that of any animal hitherto measured. *Cypridina* has a somewhat similar emission spectrum, with a maximum at *ca.* 480 mμ (Coblentz & Hughes, 1926). Polynoid light is greenish, with maximal emission at about 515 mμ (Nicol, 1957).

The biological significance of the light of *Chaetopterus* still awaits an explanation. *Chaetopterus* is light-sensitive, and presumably could detect its own light. However, the spectral sensitivity of *Chaetopterus* in unknown, so it is not possible to relate this to the colour of the luminescence. *Chaetopterus* is preyed upon by various animals, including *Limulus* and coastal fish, which may have occasion to perceive the light.

Absorption curves are available for the eye pigments (rhodopsins) of marine coastal fish, and the curves can be used to represent the spectral sensitivity of these animals (Fig. 12) (Wald, 1946; Kampa, 1953). The spectral sensitivity curve of *Limulus* has been determined (Graham & Hartline, 1935). This has a maximum at 520 mμ (Fig. 12). In Fig. 13 I have drawn curves for 'luminous flux' of *Chaetopterus* light, based on visibility values taken from the curves of Fig. 12, and on human scotopic vision (C.I.E. values). The luminous efficiency of radiation is given by the ratio of total luminous flux to total radiant flux (Harvey, 1940). For human scotopic vision, *Chaetopterus* light is 67% efficient; for fish having visual purples with maxima at 505 mμ, *Chaetopterus* light is 71% efficient; and for the eye of *Limulus*, *Chaetopterus* light is 56% efficient.

TABLE 7. SPECTRAL COMPOSITION OF THE LIGHT OF *CHAETOPTERUS*

(Correction of first approximate results)

Filter x	$\int E_{\lambda A} S_\lambda T_\lambda = \zeta_x$	$\zeta_x/\zeta_{602}=\rho_1$	$R_x/R_{602}=\rho_2$	$\rho_2/\rho_1=\rho_3$	$Q_x\rho_3$ (based on $x_{602}\equiv 100$)	Mean λ (based on curves $E_{\lambda A}S_\lambda T_\lambda$) (m$\mu$)
OV 1	1583	0·3737	0·0934	0·250	0·91	411
601	14395	3·3982	3·1027	0·913	57·72	443
602	4236	1	1	1	100	464
603	4912	1·1596	1·115	0·962	61·24	488
604	1205·4	0·2846	0·2841	0·998	40·75	513
605	451·2	0·1065	0·0909	0·854	14·86	541
606	83	0·0196	0·0187	0·954	7·54	571
607	66·6	0·0157	0·0188	0·835	3·03	585

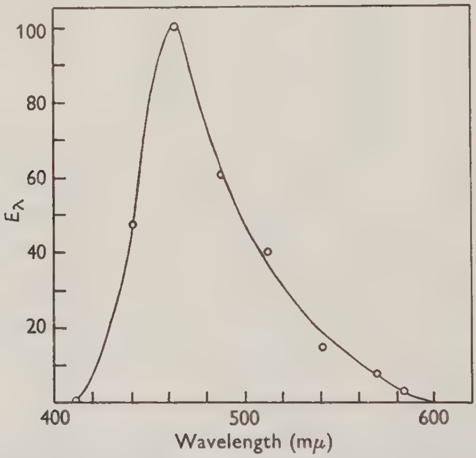


Fig. 11. Corrected values for relative spectral energy of *Chaetopterus* light, and a spectral emission curve based on these values.

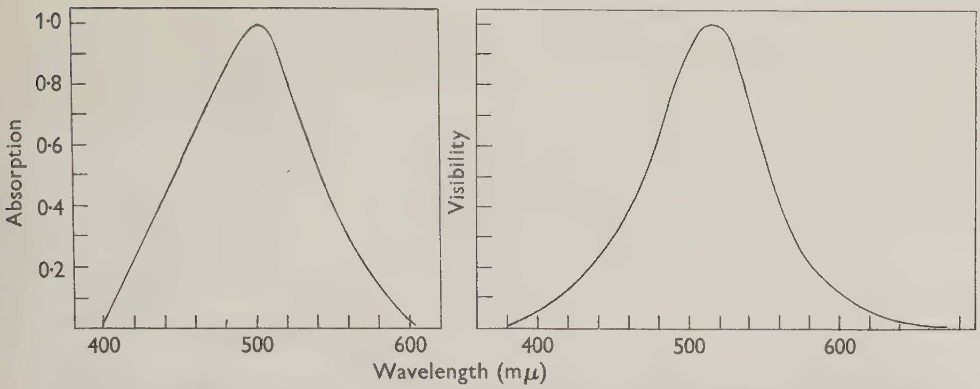


Fig. 12. Left, generalized absorption curve for visual purple of coastal marine fish (based on measurements of Wald, 1946, and Kampa, 1953). Right, visibility curve for *Limulus* (based on measurements of Graham & Hartline, 1935).

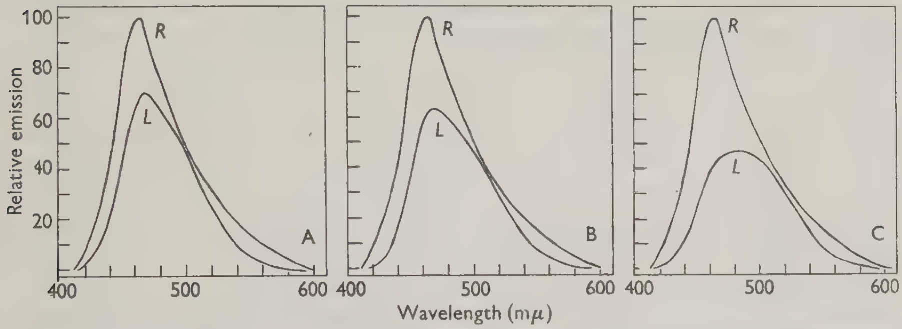


Fig. 13. Relative spectral energy curves (radiant flux) of *Chaetopterus* light, and estimated curves of luminous flux based on fish rhodopsin (A), human scotopic vision (B), and *Limulus* vision (C). *R*, radiant flux; *L*, luminous flux.

SUMMARY

The spectral composition of the light of *Chaetopterus variopedatus* has been measured by means of spectral filters and multiplier phototube. Spectral emission extends from about 405 to 605 mμ, with a maximum at about 465 mμ. The spectral curve of *Chaetopterus* light is compared with a human visibility curve (scotopic vision), a visibility curve for *Limulus*, and an absorption curve for fish visual purple. Luminous efficiencies, based on these curves, are calculated.

REFERENCES

Coblentz, W. W. & Hughes, C. W., 1926. Spectral energy distribution of the light emitted by plants and animals. *Sci. Pap. U.S. Bur. Stand.*, Vol. 21, pp. 521–34 (no. 538).

Graham, C. H. & Hartline, H. K., 1935. The response of single visual cells to lights of different wave lengths. *J. gen. Physiol.*, Vol. 18, pp. 917–31.

HARVEY, E. N., 1940. *Living Light.* Princeton University Press.

—— 1952. *Bioluminescence.* New York: Academic Press.

—— 1955. Survey of luminous organisms: problems and prospects. In: *The luminescence of biological systems*, ed. F. H. Johnson, pp. 1–24. Washington: Amer. Ass. Adv. Sci.

KAMPA, E. M., 1953. New forms of visual purple from the retinas of certain marine fishes: a re-examination. *J. Physiol.*, Vol. 119, pp. 400–09.

LANKESTER, E. R., 1868. In Harvey, 1952.

NICOL, J. A. C., 1952*a*. Studies on *Chaetopterus variopedatus* (Renier). I. The light-producing glands. *J. mar. biol. Ass. U.K.*, Vol. 30, pp. 417–31.

—— 1952*b*. Studies on *Chaetopterus variopedatus* (Renier). II. Nervous control of light production. *J. mar. biol. Ass. U.K.*, Vol. 30, pp. 433–52.

—— 1952*c*. Studies on *Chaetopterus variopedatus.* III. Factors affecting the light response. *J. mar. biol. Ass. U.K.*, Vol. 31, pp. 113–44.

—— 1957. Spectral composition of the light of polynoid worms. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 529–38.

SKOGLAND, J. F., 1929. Tables of spectral energy distribution and luminosity for use in computing light transmissions and relative brightnesses from spectrophotometric data. *Misc. Publ. nat. Bur. Stand.* No. 86, 23 pp.

WALD, G., 1946. The chemical evolution of vision. *Harvey Lect.*, Ser. 41, pp. 117–60.

J. mar. biol. Ass. U.K. (1957) 36, 643–650
Printed in Great Britain

A NEW MARINE DINOFLAGELLATE: *EXUVIAELLA MARIAE-LEBOURIAE* N.SP.

By MARY PARKE and DOROTHY BALLANTINE
The Plymouth Laboratory

(Text-figs. 1–18)

Exuviaella mariae-lebouriae n.sp., Parke & Ballantine

Exuviaella apora Schiller pro parte, in Lebour, 1925, (non *E. apora* Schiller, 1918).
Exuviaella apora Schiller, in Martin, 1929.

Diagnosis

Cell ovoid to almost sphaeroidal in face view of right or left valve, lenticular to almost sphaeroidal in view showing junction of valves, in both views flagellar pole flattened, length (10) 14–17 (22), breadth (9) 11–15 (20), thickness (5) 6–8 (18) μ. Two valves of cellulose, each with a striated band at margin, band wider in larger cells, striations about 0·5 μ apart, remainder of valve surface with poroids, apparently in rows, 0·25 μ apart, poroids 0·2–0·25 μ diameter; right valve with shallow indentation, about 2 μ wide, at flagellar pole, usually lying to left of centre line; left valve with small projecting flange, about 2 μ wide, bearing flagellar pores and fitting into and over indentation on right valve; the two flagellar pores of unequal size, in apical view larger nearly triangular, smaller circular, in left valve view smaller pore to left of larger. Two flagella, one emerging from larger pore undulating parallel to, and round, flagellar pole, 2·5–3 times body length; the other, 1·25–1·5 times body length, emerging from smaller pore. One obvious, usually ovoid, pusule, 1·5–4 μ in length, in left valve view close to surface on the right side near flagellar pole, opening to exterior by canal to larger flagellar pore; second smaller pusule sometimes present but connexion to exterior not visible. Nucleus posterior, broadly ellipsoidal in valve view. Chromatophores golden-brown, usually two in smaller cells, large, parietal, saucer-shaped with deeply lobed margins, with a single large pyrenoid on inner face of each lying in anterior part of body; in larger cells chromatophores smaller and more numerous. Disc-shaped starch grains surrounding pyrenoids and in stroma; lipoid reserve absent. Nutrition phototrophic. Trichites distributed in peripheral cytoplasm, more numerous in region of junction of valves. Non-toxic to fish.

Asexual reproduction by fission in the motile state into two daughter-cells of equal or unequal size.

Isolated from sea-water sample taken near Knap Buoy, Plymouth Sound, from 8 m depth on 30 June 1949 (type culture); also from St German's River in tow net sample on 24 June 1957. Type culture (Plymouth no. 18) at the Laboratory of the Marine Biological Association, Plymouth.

Named in honour of Dr Marie V. Lebour on the occasion of her 80th birthday.

Cellula duabus valvis (materia cellulosa consistentibus) induta, sinistra atque dextra, forma ovali vel sphaerali ut videtur pro superficie utriusque valvae, lenticulari vel fere sphaerali pro conjunctione valvarum; planata extremitate qua oriuntur flagella; longa (10) 14–17 (22) μ, lata (9) 11–15 (20) μ, crassa (5) 6–8 (18) μ; margine utriusque valvae striolata, latius in majoribus cellulis, striolis 0·5 μ separatis, valvis pro cetero perforatis minutissimis porulis latis 0·20–0·25 μ, distributis—ut videtur—in ordinibus regularibus 0·25 μ separatis; valva dextra indentata sinu haud profundo, lato fere 2 μ, ad extremitatem qua oriuntur flagella paulum sinistraliter ex norma posito; valva sinistra praedita parvo processu, eminente super sinum valvae dextrae, quem sinum idem replet. Eodem processu perforato a duobus poris ad exitum flagellorum; quibus poris disparibus magnitudine; majore dextro, fere triangulari, minore sinistro, circulari (ut videtur in aspectu sinistrae valvae). Duobus flagellis, altero emergente ex majore poro, undulante circum situm originis suae, longiore 2·5–3plo quam cellula; altero emergente ex minore poro, longiore 1·25–1·5plo quam cellula. Pusulis duabus, altera (quam semper facile potest videre) longa 1·5–4 μ, in sinistra valva posita, sub superficie, dextraliter prope originem flagellorum, aperiente externe per canaliculam in porum majorem flagelli, altero nonnunquam absente, invisibili ut aperit externe.

Legend to Figs. 1–9

Figs. 1–9. *Exuviaella mariae-lebouriae* n.sp.

(Figs. 1–2, × 5000; Figs. 3–9, × 2500)

c, chromatophore; *et*, ejecting trichite; *g*, granules in pusule; *lf*, 'longitudinal' flagellum; *m*, mitochondrion?; *n*, nucleus; *nsb*, narrow striated band at margin of valve; *p*, pyrenoid; *pu*, pusule; *s*, starch bodies; *sp*, 2nd pusule; *t*, trichite; *tf*, 'transverse' flagellum; *wsb*, wide striated band at margin of valve.

Fig. 1. Left valve view of small individual with two large chromatophores; valve shows large and small flagellar pores and a narrow striated margin.

Fig. 2. Right valve view of large individual with six chromatophores and two pusules, the larger containing granules in Brownian movement; three pyrenoids surrounded by disc-shaped starch bodies; valve shows wide striated margin and indentation at flagellar pole; the projecting flange of the left valve, bearing the flagellar pores, is seen in the indentation.

Fig. 3. View of flagellar pole showing large and small flagellar pores.

Fig. 4. Empty valves of cell showing flagellar pores on flange of left, and indentation in right, valve.

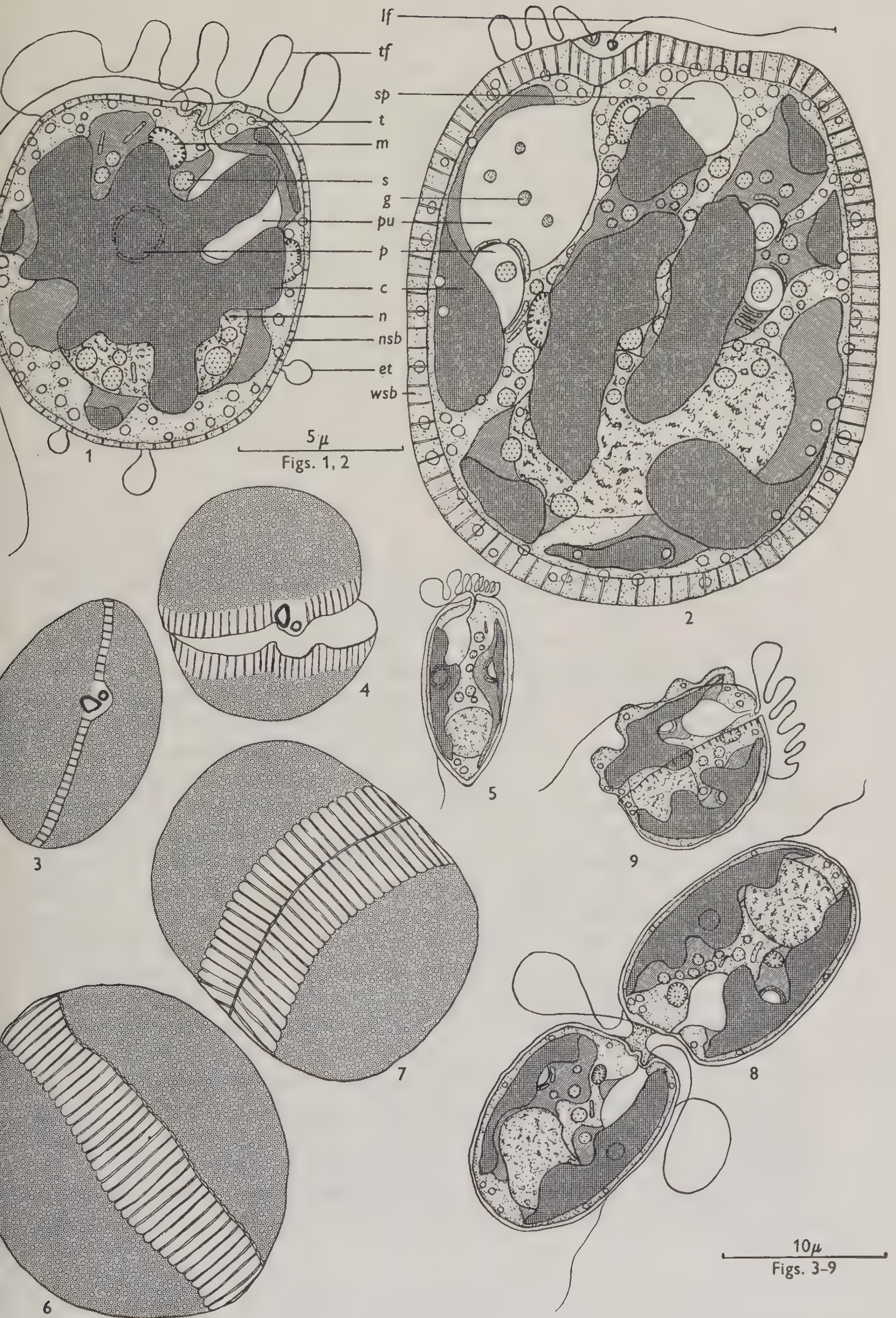
Fig. 5. Optical section of young compressed cell in side view (junction of valves—left valve on left of diagram) showing connection of pusule to larger flagellar pore.

Fig. 6. Side view of large, nearly sphaeroidal, individual with the wide striated margins to the valves still overlapping.

Fig. 7. Early fission stage with valves not overlapping; the wide striated margins of both valves are clearly visible.

Fig. 8. Late fission stage: daughter-cells still joined by narrow connexion near flagellar pole.

Fig. 9. Newly separated daughter-cell with one valve.


lf
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10μ
Figs. 3–9

Figs. 1–9

Nucleo posteriore posito, late ellipsoideo ut videtur per superficiem utriusque valvae; chromatophoris aureo-brunneis, in nonnullis minoribus cellulis ex norma duobus, magnis, prope cellulae parietem positis, crateriformibus, margine profunde lobatis, quoque chromatophoro magnum corpus pyrenoideum in aspectu interno continente, versus partem anteriorem cellulae positum; in nonnullis majoribus cellulis chromatophoris minore magnitudine, numerosioribus. Granulis amylosis circum corpora pyrenoidea et in stromate distributis; materia lipoidea absente. Nutritione phototrophica. Corporibus trichiformibus distributis in cytoplasmate superficiali, numerosioribus prope conjunctionem valvarum. Cellula non toxica piscibus.

Generans asexualiter per fissionem cellularum motilium ad duas cellulas filiolas pares vel impares magnitudine pariendas.

Isolata exemplis aquae marinae prope Knap Buoy, regione Plymouth Sound, profund. 8 metr., 30 jun. 1949. Cultura typica conservata (Plym. num. 18) ad Laboratorium Marine Biological Association, Plymouth.

Species dedicata in honorem Mariae V. Lebouriae, doctoris scientiarum, die natali suo octogesimo.

We wish to thank Dr J. E. Morton (Queen Mary College) for translating the English diagnosis into Latin.

Description

Cells are strongly compressed when young (Fig. 5), but gradually increase in size and thickness by the growth in width of the striated band at the edge of each valve (Figs. 1–3, 6); sphaeroidal cells are usually incipient fission stages. The striated edges of the valves appear thicker than the poroid region, and the striae apparently alternate on the two valves (Fig. 7). The striated band of one valve completely overlaps the other until the incipient fission stage is reached (cf. Figs. 6, 7). In the young stage the flange on the left valve, bearing the flagellar pores, is very little developed, and the pores lie in the poroid region. As the cell increases in size the flange becomes more obvious since a region of non-striated material, equivalent to the width of the striated margin, is laid down between the poroid region of the valve and the flagellar pores (Fig. 4); the flagellar pores therefore remain near the edge of the flange.

The position of the flagellar flange on the left valve is variable. When observed in left valve view it is usually situated to the right of the centre line (Fig. 1), but depending on the shape of the valves may be on, or even slightly to the left of, the centre line. Whatever the position of the flange on the left valve, the smaller (*ca.* 0·5 μ) circular pore is always to the left of the larger (*ca.* 1·5 μ) triangular pore (Figs. 1–4) (cf. *Porella perforata*, Braarud, 1945).

As the cells increase in size and thickness the two chromatophores break up, giving from four to six disc- to ribbon-shaped chromatophores (Fig. 2). Individual chromatophores are then difficult to distinguish as they overlap and intertwine round the many other cell contents. The two pyrenoids, one on the inner face of each chromatophore in the more compressed cells (Figs. 1, 5), can be more easily seen than those (usually four) in the thicker larger cells (Fig. 2). The addition of extremely dilute iodine will, however, show up the pyrenoids more clearly by staining them orange-brown. This dilute iodine also stains blue the very numerous disc-shaped bodies, up to 1 μ in diameter, which we take to be starch (Figs. 1, 2 and 5). They surround the pyrenoids and are also freely distributed in the cytoplasm. Both pyrenoids and starch grains are masked by the staining of other cell contents when strong iodine is used. No lipoid reserve has been detected by the use of osmium tetroxide, Sudan black and Sudan IV.

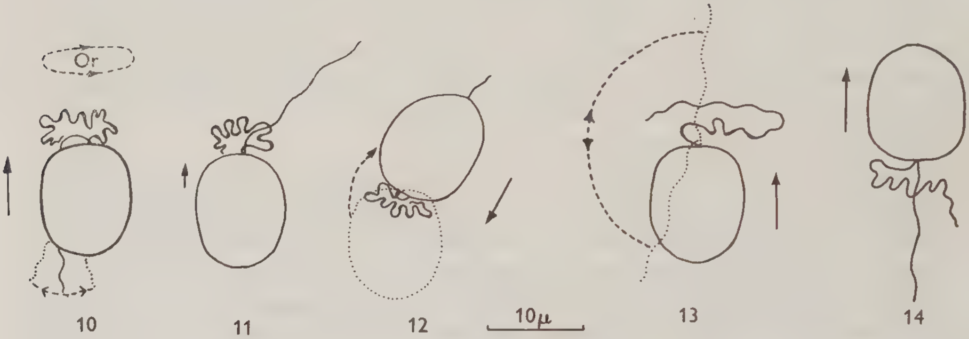
Tests with graphite (cf. Parke, Manton & Clarke, 1955, p. 582) have given no evidence of phagotrophy. Minute granules (about 0·5 μ) showing Brownian movement have, however, been found in the large, but not in the small, pusule (Fig. 2). The origin of these granules is unknown. The large pusule (Figs. 1, 2 and 5), which opens into the large flagellar pore, can be seen to increase or decrease in size. The contents are immediately exuded as globules through this pore when the undulating flagellum is cast. In life, under the lower powers of the microscope (up to 8 mm objective), the contents of the pusule appear a very pale pink-fawn, but colourless under higher powers. With cresyl or methylene blue the contents stain pink-violet.

Two ovoid bodies, about $1{\cdot}5 \times 1\,\mu$ in size, staining with Janus green are possibly mitochondria (Figs. 1, 2). As in many Dinophyceae (Chadefaud, 1936, p. 327; Chatton, 1952, p. 313) and Chrysophyceae (Magne, 1954; Parke *et al.*, 1955, 1956) this species possesses ejectile organelles (trichites). In the living cell they appear (Figs. 1, 2) as refractile spheres about 0·5 μ in diameter, and can exude their contents through the poroids as globules, batons or threads (Fig. 1). With cresyl or methylene blue they stain a deep clear blue (cf. Chrysophyceae, Parke *et al.*, 1955, 1956).

During swimming the undulating flagellum ($\equiv$ transverse flagellum of dinoflagellates possessing a girdle) always moves parallel to, and round, the flagellar pole, only the speed and amplitude of the undulations varying. The position of the so-called longitudinal flagellum varies with the speed of movement of the cell. During rapid swimming it is backwardly directed and vibrates quickly (Fig. 10). Movement of the cell is in an irregular double spiral with many changes of direction. When the cell changes direction the 'longitudinal' flagellum sweeps rapidly forward, the cell stops and the body immediately turns over to lie on the 'longitudinal' flagellum (Figs. 10–12). The cell then swims away in the new direction. Larger, more sphaeroidal, cells rotate relatively more slowly with a more rolling motion. During slow movement there is no rotation of the body. The cells usually move with the flagellar pole foremost, and with the 'longitudinal' flagellum either directed backwards, and not apparently moving, or sweeping slowly backwards and forwards in a half circle (Fig. 13). Occasionally the cell moves with the non-flagellar pole foremost (Fig. 14) for short periods. Although no stigma can be detected, this species shows a phototactic reaction when exposed to uneven illumination.

At the peak of growth a culture of this species in Erdschreiber solution will produce about 200,000 cells per ml., which is close to the production of cells (160,000/ml.) in a culture of *E. baltica* Lohmann (Plymouth no. 28).

Cell division takes place in the motile state. As the incipient fission stages gradually increase in thickness, the two valves move apart. At first the wide striated region of the valves is seen as two distinct bands (Fig. 7). After this stage these broad bands are not visible, and there appears to be an area of naked protoplasm between the two valves (Fig. 15). Division of the nucleus then occurs and the two new flagella are formed (Figs. 15, 16). Fission of the body starts at the non-flagellar pole and proceeds until the two daughter-cells are joined by only a narrow connexion near the flagellar pole (Figs. 8, 16–18).



Figs. 10–14. *Exuviaella mariae-lebouriae* n.sp. (× 1250). Cells showing positions of flagella in relation to the body during swimming. Arrows show direction of movement.

Fig. 10. 'Longitudinal' flagellum backwardly directed, vibrating rapidly, as in fast movement.

Fig. 11. 'Longitudinal' flagellum sweeps forward, cell slows down to change direction.

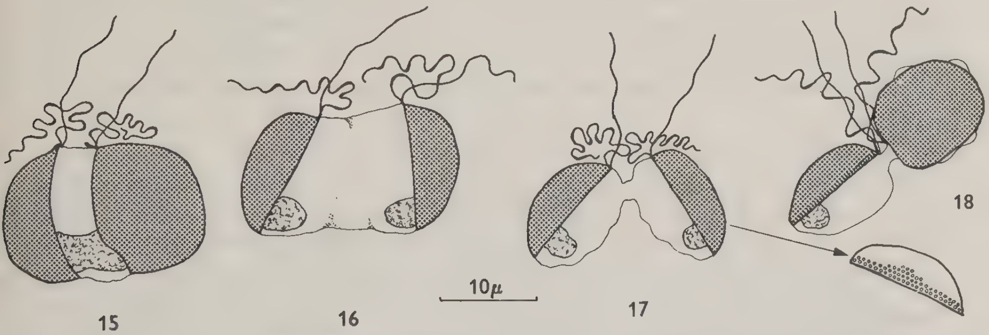
Fig. 12. Cell-body turns over to lie over the 'longitudinal' flagellum, to complete the change of direction.

Fig. 13. Cell showing action of flagella in slow movement with flagellar pole foremost. 'Transverse' flagellum moves with looser and slower undulations.

Fig. 14. Slow movement with non-flagellar pole foremost.

The two daughter-cells swim joined together for some time, then break apart. Each daughter-cell usually retains one valve and secretes one new one. Small cells with one valve and the remainder of the body showing a lobed outline are seen frequently (Fig. 9). Examination of the valves on late fission stages and newly separated daughter-cells shows that they either lack, or have only very narrow striated margins (Figs. 9, 17); the flange bearing the flagellar pores is also very small (see p. 646). It is thought that the prominent flange and the wide striated margin of the incipient fission stage is either cast or resorbed during fission. If this is the case both daughter-cells will develop new flagellar pores and this is consistent with the evidence obtained from the examination of the valves of young specimens.

Of the species which show a superficial resemblance to *E. mariae-lebouriae*, *E. compressa* Ostenfeld and *E. pusilla* Schiller differ in having only one flagellar pore; they can also be distinguished by other characters. *E. cordata* Ostenfeld appears rather similar but has a depression at the flagellar pole and has thick-walled valves which apparently lack striated margins. *E. baltica* Lohmann is similar to *E. mariae-lebouriae* in having two flagellar pores and striated margins to the valves (Woloszynska, 1928; also Plymouth Culture no. 28—private communication from Professor T. Braarud and our own observations using a light microscope). *E. baltica* differs, however, in being smaller and almost spherical, in having smaller flagellar pores, in lacking poroids and in the chromatophores being much more distinct and more deeply pigmented, although both species produce starch as a reserve product. *E. apora* Schiller (1918, p. 258) is larger (30–32 μ × 21–26 μ) than *E. mariae-lebouriae* and the valves lack poroids. In the original diagnosis of *E. apora* there is no mention of the edges of the valves being striated, nor is the number of flagellar pores given. Lebour (1925) placed specimens (16–22 μ long) found near Plymouth in Schiller's *E. apora*, but states: 'It is possible that there are here two separate species....' In these specimens she records the presence of two flagellar pores and striated margins in the older cells. She also records the breaking up of the two chromatophores into small spheres (cf. Martin, 1929, p. 10). No poroids in the valves were observed by Dr Lebour, but these can be seen only with critical brightfield illumination and objectives of very high resolution or



Figs. 15–18. *Exuviaella mariae-lebouriae* n.sp. (× 1250). Stages in cell division (see also Figs. 7–9). Valves are indicated by stippled area.

Fig. 15. Early fission stage: the two new flagella are formed and the valves lie apart, leaving an area of naked protoplasm; the broad striated bands at the margins of the valves of incipient fission stages cannot be seen.

Fig. 16. Nucleus has divided, and cell begins to show indentations at poles.

Fig. 17. Later stage of fission, showing position of valves. The inset diagram of one valve shows the poroids extending to the margin of the valve, i.e. striated area is lacking.

Fig. 18. Last stage of fission before daughter-cells break apart; one daughter-cell has twisted round with respect to the other, showing one valve in face view; new valves are not yet formed.

with dark-ground illumination using a 4 mm objective. We are certain that our material is identical with that described and figured by Dr Lebour and therefore designate her Plymouth specimens, placed by her in *E. apora* Schiller, as *E. mariae-lebouriae* n.sp. (non *E. apora* Schiller). Martin (1929) records *E. apora* from New Jersey and states he has 'no hesitation in applying this name in Lebour's sense to our material. Whether it is the same as Schiller's original species is less certain.' In his description Martin mentions that a small spine is sometimes present on the left valve but that it is seen with difficulty and only in certain positions. We have not observed spines on the left valve in our material, but in some specimens the margin of the larger pore is raised more than in others and occasionally gives the impression of being a small spine when seen from certain angles. Schiller (1933) revised his diagnosis of *E. apora* to include Dr Lebour's observations. This later description therefore includes both species and requires emendation.

REFERENCES

Braarud, T., 1945. Morphological observations on marine dinoflagellate cultures (*Porella perforata*, *Goniaulax tamarensis*, *Protoceratium reticulatum*). *Avh. norske VidenskAkad., M.-N. Kl.* 1944, no. 11, 18 pp.

Chadefaud, M., 1936. Les Protistes trichocystifères ou Protogastréades. *Ann. Protist., Paris*, T. 5, pp. 323–41.

Chatton, E., 1952. Classe des Dinoflagelles ou Péridiniens, in *Traité de Zoologie*, Ed. P. P. Grassé, T. 1, Fasc. 1, 1071 pp. Paris.

Lebour, M. V., 1925. *The Dinoflagellates of Northern Seas.* 250 pp. Plymouth.

Magne, F., 1954. Les Chrysophycées marines de la Station biologique de Roscoff. *Rev. gén. Bot.*, T. 61, pp. 389–415.

Martin, G. W., 1929. Dinoflagellates from marine and brackish waters of New Jersey. *Stud. nat. Hist. Ia Univ.*, Vol. 12, no. 9, pp. 1–32.

Parke, M., Manton, I. & Clarke, B., 1955. Studies on marine flagellates. II. Three new species of *Chrysochromulina*. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 579–609.

—— —— —— 1956. Studies on marine flagellates. III. Three further species of *Chrysochromulina*. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 387–414.

Schiller, J., 1918. Über neue *Prorocentrum*—und *Exuviella*—Arten aus den Adria. *Arch. Protistenk.*, Bd. 38, pp. 250–62.

—— 1933. Dinoflagellatae (Peridineae). *Rabenhorst's Kryptogamenflora*, Bd. 10, Abt. 3, Teil 1.

Woloszynska, J., 1928. Dinoflagellatae Polskiego Bałtyku i Błot nad Piaśnicą. *Arch. Hydrob. Rybact.*, T. 3, pp. 153–278.

J. mar. biol. Ass. U.K. (1957) 36, 651–662
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THE PHOTOSENSITIVE PIGMENTS IN THE RETINAE OF DEEP-SEA FISH

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(Text-figs. 1 and 2)

The deep-sea fishes might be expected to have eyes specialized for their environment because this differs in some striking respects from that of terrestrial and shallow water animals. The groups of mid-water oceanic fish on which most of these experiments were made live in the 'twilight' zone of the ocean. Here daylight, much attenuated by the sea water through which it has passed, is blue-green in colour, and the light given by the luminescent organs of animals is an important fraction of the total light available (Clarke & Wertheim, 1956). These animals probably have two visual tasks, the first that of perceiving the daylight above by which they must regulate their daily vertical migrations, and second seeing the small flashing photophores of other animals or the small shadows of other animals against daylight which serve to guide them to their food and their mates and to warn them of the presence of enemies. These two tasks do not demand the same kind of eye.

To see small spots of light efficiently a large collecting pupil is needed, and a large eye such as the eye of an oceanic squid, provided that it can form a good image, will have very distinct advantages over a small one. To see a very large field such as that given by the daylight coming through sea water the size of the eye is not important, but only the aperture, i.e. a relationship between size of pupil and the other dimensions of the eye. But whatever the structure and use of the eye the efficiency with which the retina absorbs the light quanta incident on it will be of the first importance. The light penetrating oceanic waters best is blue-green in colour, and the deeper the ocean is penetrated the more and more the penetrating light becomes confined to a narrow band of wavelengths around 475 μ (Jerlov, 1947–8). This fact has suggested to Marshall (1954) that the eyes of deep-sea fish would be especially sensitive to such blue light, a prediction which has been borne out by experiment (Denton & Warren, 1956).

METHODS

The eyes of many deep-sea fish are quite small and are not in general available in such numbers as to make the classical methods of extraction useful. The measurements were therefore made on intact retinae using new methods described in detail elsewhere (Denton & Wyllie, 1955; Denton, 1954; Denton & Walker, 1957) which demand only very small areas of retina. Such methods have the added advantage of giving directly the density of pigment in the retina, a quantity essential to an evaluation of the possible sensitivity of the eye. The method is briefly as follows: (1) Under Ringer's solution the retina is dissected from the dark adapted eye. It is floated rod-side uppermost into a chamber made by fixing a ring of wax on to a microscope slide and then, after filling with Ringer's solution, this chamber is closed with a cover glass. (2) An image of the retina is thrown by a $\frac{2}{3}$ in. objective on to a diaphragm and the retina moved until the image of a uniform part of the retina in which the rods are upright is seen to fall on the hole in the diaphragm. The eye of the observer is replaced by a photomultiplier tube which thus collects light which has passed through one thickness of a small area of retina. (3) The colour and intensity of the light reaching the retina can be changed by neutral and colour filters placed between the light source and the substage condenser of the microscope. The output of the photomultiplier is measured with a Cossor oscillograph (Model 1049). (4) Measurements are made for a series of colour filters both before and after bleaching the retina with strong white light. The assumption is made (Denton & Walker, 1957) that the retina contains no photosensitive pigment appreciably absorbing deep red light of wavelength about 650 mμ, and that this light can be used as a reference light showing changes in sensitivity of the light source and recording system. Corrections are thus calculated which are used for measurements with other colour filters. The apparatus is very robust and, since the measuring instrument is virtually without inertia, it can be used aboard ship without any special difficulty. The apparatus was not appreciably disturbed by the movement of the ship in any but the heaviest seas.

MATERIAL

The experiments were made on freshly caught deep-sea fish; some aboard R.V. *Sarsia*, and others aboard R.R.S. *Discovery II*. Aboard R.V. *Sarsia* the fish were caught in the Bay of Biscay off the continental shelf by verticals hauls made from 2000 m with a 2 m stramin ring trawl. Aboard R.R.S. *Discovery II* the fish were caught in an Isaacs Kidd mid-water trawl fished at various depths estimated at being between 200 and 1500 m. The catch was generally brought up either at twilight or after dark and care taken not to expose the fish used for experiments to bright lights. Some of the fish used were alive when brought to the surface but others were dead. Since the haul with the Isaacs Kidd trawl was generally for 4 h it is possible that some fish had been dead for 4 h before experiments were begun.

RESULTS

EFFECT OF TIME ON THE DIFFERENCE CURVE

On bleaching a retina by exposing it to a bright light a chain of reactions is started which continues in the dark. These reactions were first described by Kühne (1878) in his classical work on visual purple. The retina of the frog, which Kühne used extensively, is rose pink in colour when unbleached and passes, following exposure to a bright light, through orange and yellow to become finally colourless. Similarly, following bleaching, the photosensitive pigments in the retinae of deep-sea fish, which are all golden in colour when unbleached, undergo a series of reactions in which the products have absorption spectra displaced progressively farther towards the ultra-violet. This is shown in Figs. 1 A and B, in which difference curves (a difference curve shows the change in density between unbleached and bleached retinae as a function of wavelength) at various times following bleaching are given. No matter how long a time is given after bleaching, the products of bleaching are always appreciably more heavily absorbing in the deep blue and near ultra-violet than the unbleached pigment from which they were formed. This is very characteristic of this group of golden pigments. Rhodopsin and porphyropsin pigments in the intact retina usually give products of bleaching in which the absorption in the deep blue and near ultra-violet is very like, or less, than that of the unbleached pigment.

For the deep-sea fish the dark reactions go on very slowly after the first hour following bleaching. Most of the difference curves given here are of difference in density between an unbleached retina and the retina an hour after bleaching.

EFFECT OF TIME AFTER DEATH OF THE ANIMAL AT WHICH MEASUREMENTS ARE BEGUN

To find the possible effect of the death of an animal for a period of 4 h before observations are made (4 h was the time of a typical haul), measurements were made on the retinae of a *Chauliodus sloanei* which was caught alive. One retina was dissected and used immediately after death, whilst the other retina was dissected and used 4 h later. Fig. 1 C shows that the difference curves are very similar except that the dark reactions following bleaching have gone less far in the later preparation. The consequent distortion of the curve is very slight over most of the visual spectrum and insufficient to displace the wavelength of maximum difference in density.

The maximum density change on bleaching was smaller for the 'older' preparation. The maximum density change for the first retina being 0·67 and of the second 0·40. It was the rule in these experiments that high retinal densities were always found in those fish caught alive.

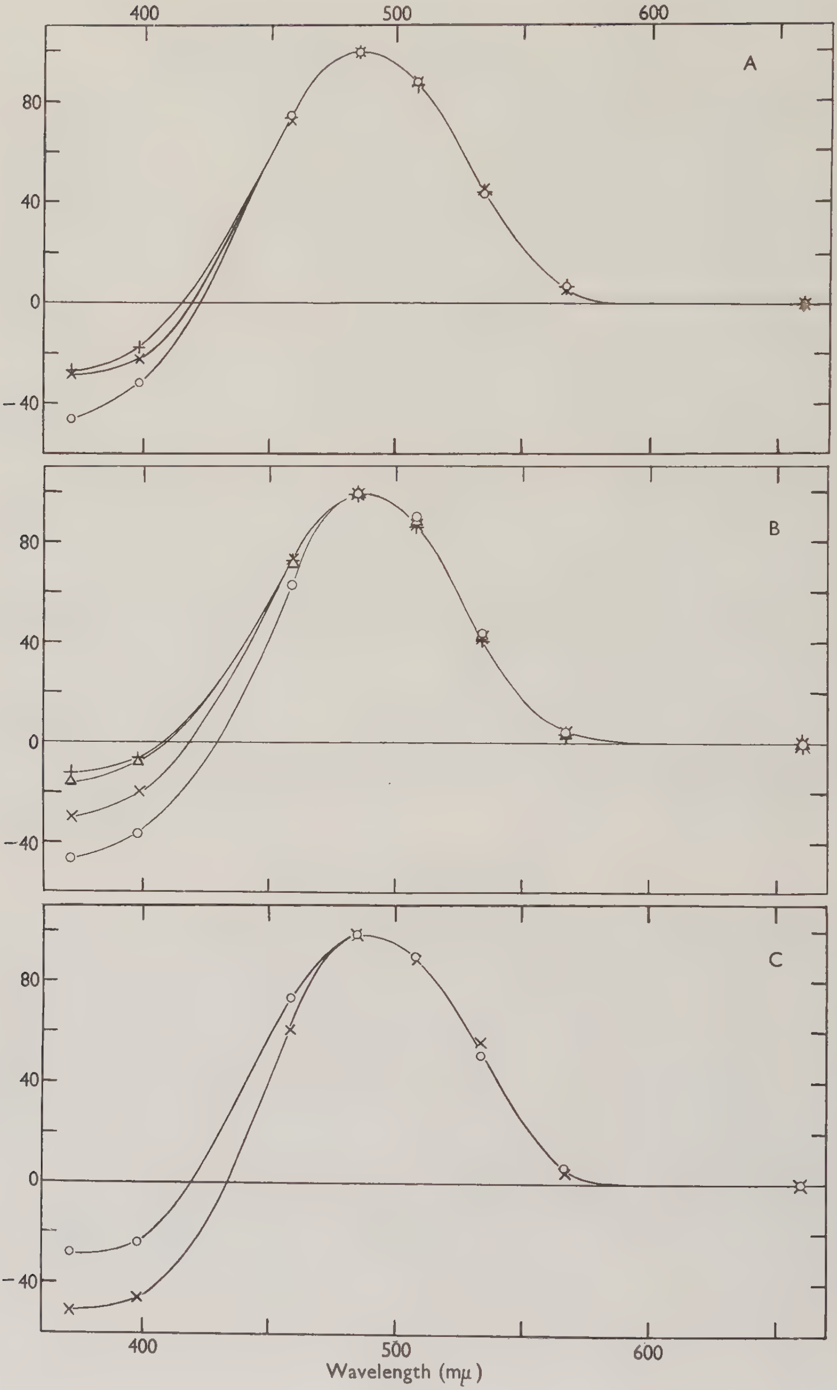
400
500
600
A
80
40
0
−40
B
80
40
0
−40
C
80
40
0
−40
400
500
600
Wavelength (mμ)

Fig. 1

DENSITY OF PIGMENT

The density change on bleaching was measured for all the preparations used (Table 1). The maximum density change was always for light of wavelengths around 485 mμ. When the measurements were completed it was always possible to see the small area of colourless bleached retina in the otherwise golden-coloured retina, and thus confirm that only one thickness of retina had been used for the measurements on which the density change is based.

TABLE 1. DENSITY CHANGE ON BLEACHING FOR LIGHT OF WAVELENGTH 485 Mμ

Family	Species	Max. density change
Melamphaidae	*Melamphaës megalops* Lütken	0·19
,,	*Melamphaës unicornis* Gilbert	0·56
Synaphobranchidae	*Synaphobranchus kaupi* Johnson	0·27
Sternoptychidae	*Argyropelecus hemigymnus* Cocco	1·08
,,	*Argyropelecus aculeatus* C.V.	1·20*
,,	*Argyropelecus olfersii* (Cuvier)	0·78
Chauliodontidae	*Chauliodus sloanei sloanei* Schneider	0·67
,,	*Chauliodus danae* Regan & Trewavas	0·46
Myctophidae	*Lampanyctus ater* Tåning	0·31
,,	*Diaphus rafinesquei* (Cocco)	0·43
,,	*Lampadena braueri* Zugmayer	0·21*
,,	*Myctophum punctatum* Rafinesque	0·41
Gonostomatidae	*Gonostoma elongatum* Günther	1·01
Searsidae	*Searsia* sp.	1·21
Diretmidae	*Diretmus argenteus* Johnson	0·98*
Argentinidae	*Opisthoproctus soleatus* Vaillant	0·54
Stomiatidae	*Stomias boa* (Risso)	0·79
Melanostomiatidae	*Flagellostomias* sp.	0·58

* For these retinae only the density change at 485 mμ was measured and not the difference curve.

To these we may add *Conger vulgaris*, 0·58 (see Denton & Walker, 1957).

Of the oceanic fish examined only one, a surface-living fish, *Scombresox saurus*, had a rose-coloured retina. The maximum density change for its retina was at 508 mμ and was 0·60 and the difference curve typical of the coastal fish.

SHAPE OF ABSORPTION CURVES

Parts of all retinae were examined visually in white light. All were found to have golden-coloured pigments except the gar fish (*Scombresox saurus*), which was rose-coloured, and the myctophid *Diaphus rafinesquei* (Cocco), which was

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Legend to Fig. 1

Fig. 1. Curves show difference in density between unbleached and bleached retina plotted against wavelength. The maximum fall in density on bleaching is made equal to 100%. A, *Argyropelecus hemigymnus* Cocco: ○——○, 4–20 min after bleaching; ×——×, 35–45 min. after bleaching; +——+, 60–70 min after bleaching. B, *Gonostoma elongatum* Günther: ○——○, 1 h after bleaching; ×——×, 2 h after bleaching; △——△, 6 h after bleaching; +——+, 24 h after bleaching. C, *Chauliodus sloanei sloanei* Schneider, showing the effect of death of animal on the density difference curve between unbleached and bleached retina. The measurements on bleached retina were made 1 h after bleaching. ○——○, retina dissected immediately after death; ×——×, retina dissected 4 h after death.

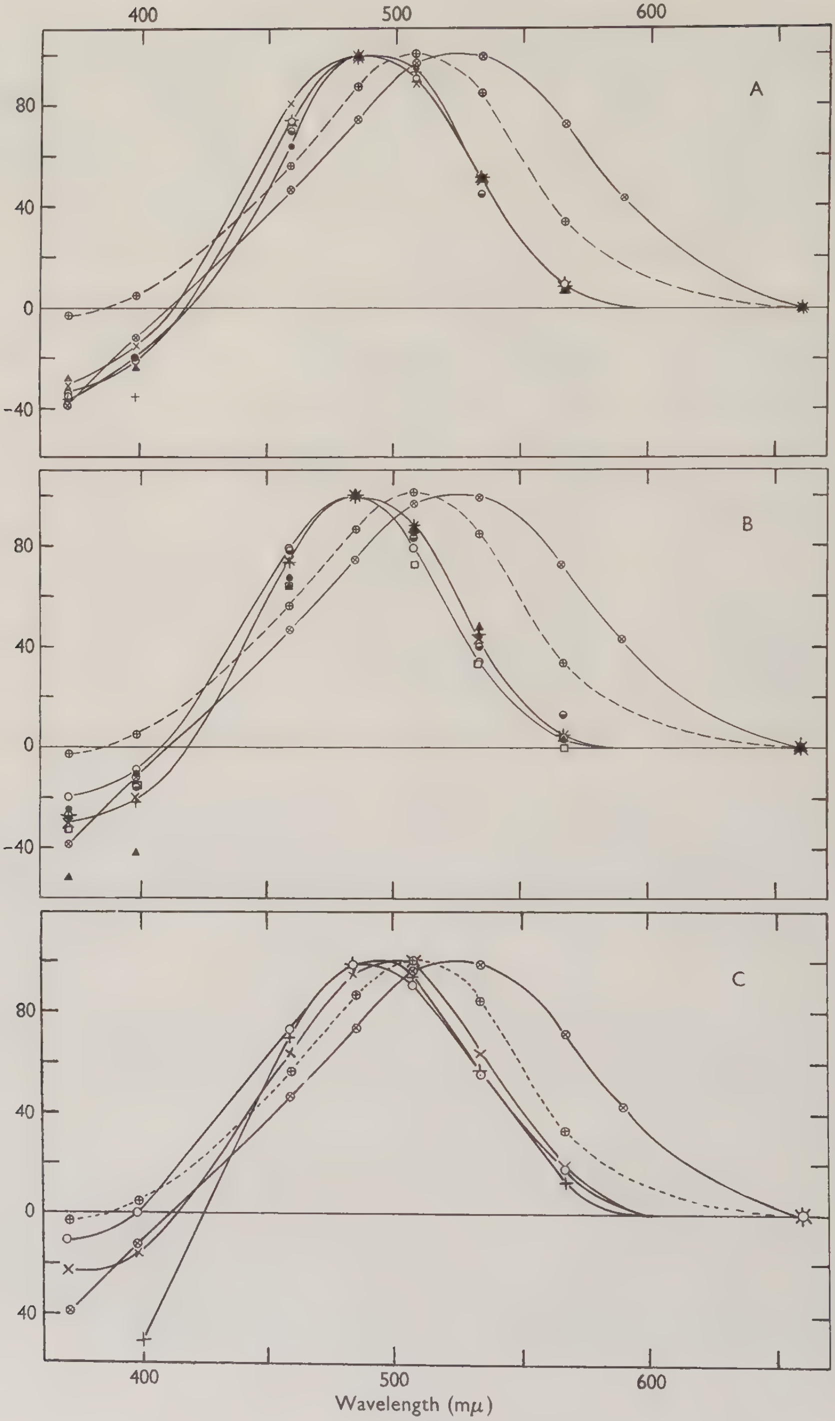

Fig. 2

noted as having a colour intermediate between that of the conger and the gar-fish, and was later found to give an absorption curve that fell between those for these two fish.

The results for the fish are plotted in three groups: (1) the difference curve very closely resembles that of the conger eel (Fig. 2A); (2) the maxima of absorption are displaced a little farther towards the blue than the conger eel (Fig. 2B); (3) the maxima of absorption are displaced a little farther towards the red than the conger eel (Fig. 2C).

One other species, *Argyropelecus aculeatus* C.V., was examined visually and found to have a golden-coloured retina, but absorption curves were not measured on it.

DISCUSSION

A good number of visual photosensitive pigments are now known and recent work, particularly by Dartnall and his collaborators, has shown that the types of scotopic visual pigments are more varied than was at first thought. The broad generalization, however, made by Wald (1945–6) that freshwater fish have retinae containing principally purple-coloured pigments (porphyropsins), whilst the coastal marine fish have retinae containing principally rose-coloured pigments (rhodopsins) remains substantially true. As a result of the present experiments it seems likely that the great majority of deep-sea fish, if not all, have retinae containing principally golden-coloured pigments and thus form a third great grouping. Denton & Warren (1956) have suggested the names chrysopsins or visual golds for these golden-coloured pigments. On the basis of the experiments described here we cannot eliminate the possibility that some of these golden-coloured retinae contain mixtures of pigments. [Miss M. A. Walker (private communication) finds that a digitonin extract of the retinae from six *Diaphus rafinesquei* (Cocco) caught in the same haul as the one used in the experiment described above, contained in fact only one photosensitive pigment.]

The difference curves shown in Fig. 2 for deep-sea fish differ from those measured on rhodopsin and porphyropsin retinae in that the increase in density

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Legend to Fig. 2

Fig. 2. Curves showing density difference between unbleached and bleached retinae plotted against wavelength. The maximum fall in density for each retina is made equal to 100%. A, Curves for the following species: ○—○, *Conger conger*; ×—×, *Myctophum punctatum* Rafinesque; +, *Flagellostomias* sp.; ●—●, *Melamphaës megalops* Lütken; ▲, *Chauliodus sloanei sloanei* Schneider; ◒, *Opisthoproctus soleatus* Vaillant. For comparison: ⊕---⊕, *Cottus bubalis*; ⊗—⊗, sea trout. B, Curves for the following species: ○—○, *Argyropelecus olfersii* (Cuvier); +, *Argyropelecus hemigymnus* Cocco; ×—×, *Gonostoma elongatum* Günther; ▲, *Chauliodus danae* Regan & Trewavas; ●, *Melamphaës unicornis* Gilbert; ◒, *Searsia* sp.; ■, *Synaphobranchus kaupi* Johnson. For comparison: ⊕---⊕, *Cottus bubalis*; ⊗—⊗, sea trout. C, Curves for the following species: ○—○, *Lampanyctus ater* Tåning; +—+, *Stomias boa* (Risso); ×—×, *Diaphus rafinesquei* (Cocco). For comparison: ⊕---⊕, *Cottus bubalis*; ⊗—⊗, sea trout.

on bleaching for wavelengths between 380 and 400 mμ is much greater. This characteristic, which they share with the conger eel and the silver freshwater eel (Carlisle & Denton, unpublished observations) and which must represent a difference in the course of the dark reactions following bleaching, gives another reason for grouping the pigments of these fish together.

Wald (1938–9) has shown that the colour of the principal retinal photosensitive pigment of a freshwater or coastal marine fish is correlated with the fishes' enviroment (more exactly that in which it spawns) rather than with its family affiliations. Here again, for the deep-sea fish, we find fishes of the most varied appearance, habits and families sharing the characteristic of golden-coloured retinae, a property which they share with no other vertebrates so far studied.

The limit to the depth at which daylight could be detected

The known ways of assessing the sensitivity of an animal's eye, that of studying the animal's behaviour or by recording the change in nervous activity of the eye when lights are flashed into it, are not easily used on deep-sea fish, which are difficult to keep alive for more than a few hours and then only aboard a ship whose movements would make electrophysiological recording almost impossible. Even when such methods can be used it is difficult to be sure that behavioural responses are really made at the limit of the animal's sensitivity or that electrophysiological recordings are made from the most sensitive receptor and nervous elements. In this paper the method of approach is that of comparing the eyes of the deep-sea fish with our own and trying to assess what advantages and disadvantages may be given to them by any peculiarities of their eyes in the particular environment in which they live. Clarke (1936) has made estimates of the same type using the known sensitivity of the sun-fish (*Lepomis*) as the starting-point for his calculations.

Clarke & Wertheim (1956) give the energy of penetrating daylight at 610 m (the remaining daylight will effectively all be of wavelength around 475 mμ) as 10^{-6} μW cm^2, which corresponds to a flux of 10^{-5} ergs/sec/cm^2. The incident flux required at the eye for the human just to see a large uniform field of this wavelength is given by Denton & Pirenne (1954) as 10^{-7} ergs/sec/cm^2 of retina, or approximately 4×10^{-7} ergs/sec/cm^2 at the pupil. This would imply that at 610 m in sunlight, in the part of the ocean where Clarke & Wertheim made their measurements, the penetrating sunlight for an observer looking upwards would be about × 25 above threshold. The oceanic waters of Clarke & Wertheim reduced penetrating daylight (at these depths only blue-green light is left) to about 10% for approximately every 120 m and the absorption of oceanic water would, therefore, set the depth at which daylight could just be detected by the human eye at about 950 m. The value given by direct observation by Beebe (1935) is about 550 m, but this refers to daylight in a horizontal sense since the windows of his bathysphere faced towards its side.

Light cannot be effective for vision unless it is absorbed by the photosensitive pigments of the retina, and in this respect the eyes of the deep-sea fish probably have considerable advantages over our own. These include (1) the pupils of deep-sea fish are, relative to the other dimensions of the eye, very much larger than human pupils. For the conger we can easily calculate (see Le Grand, 1948) that the gain in retinal illumination due to this factor is about × 2·5. It would be difficult to calculate the gains for some fish, e.g. *Chauliodus sloanei*, because the crystalline lens does not fill the pupil, but we can easily show that even an eye with no dioptric system and with light falling directly on to the photosensitive surface the gain would be × 5, and this is the maximum possible gain. (2) The eye media of the human eye absorb about 50% of light of 500 mμ as it passes from the cornea to the retina. This absorption will almost certainly be much less in the eye media of deep-sea fish because the lenses can be seen to be very transparent and the eyes are very much smaller than human eyes. This may give the deep-sea fish a gain of × 2 in retinal illumination over the human. (3) The density of retinal photosensitive material is usually very much greater in the deep-sea fish than the human. Rushton (1956) gives the human retinal density of rhodopsin for light of 500 mμ as about 0·15, while we find that retinal densities of 1·0 or more are not uncommon in deep-sea fish. This will mean that the human retina will absorb about 30% of blue-green light incident on it whilst the deep-sea fish will absorb about 90% of blue-green light incident on it, thus giving the deep-sea fish a gain of × 3 over the human.

Taking these three factors we may suppose that if a deep-sea fish and a human were both looking at the same large field of blue-green light, the number of quanta absorbed/cm^2 of retina/sec would be between 15–30 times greater for a deep-sea fish than for the human.

This suggests that if man and deep-sea fish are equally efficient at extracting information from absorbed quanta, then the deep-sea fish would see daylight at depths between 130 and 170 m deeper than could man. This would put the maximum depth at which it could see daylight at about 1100 m.

Light of a given wavelength can only be absorbed in packets of energy corresponding to the energy hv of the quantum of that wavelength (h is Planck's constant and v is the frequency of the light waves). The energy of the quantum, for light of 475 mμ, the blue-green light which penetrates oceanic waters best (Jerlov, 1947–8), is about 4×10^{-12} ergs. Even at the absolute threshold for the human eye when looking at a large field the number of quanta *absorbed* by the retinal photosensitive pigments is quite large; Denton & Pirenne give the value of 3000 quanta/cm^2 of retina/sec, a number which leaves a very large margin for improvement in the efficiency of detection. This limit to vision may be set by spontaneous activity in the retina which, to use the jargon of radar, constitutes a 'noise' amongst which the 'signal' of the external light must be detected. Now a likely site of spontaneous activity seems to be the spontaneous breakdown (in the dark) of the visual photosensitive molecules within the rods which, since light acts by breaking down the molecules, would give rise to nervous activity of exactly the same type as that given by a dim background light. The rate at which human visual purple would have to break down in the dark in order to be the limiting factor in the detection of light, was calculated as being 0·0005 %/h by Denton & Pirenne. We know that in extracts with digitonin it breaks down at the much higher rate of 1 %/h (St George, 1952), whilst Barlow (1956, 1957) has calculated the consequences of the idea that retinal 'noise' sets the limit to visual performance and has, moreover, shown experimentally that many of the predictions of his theory hold good. Reconsidering the possible gain in sensitivity of the deep-sea fish over the human with the idea of retinal 'noise' in mind, we have as before the same advantages because of the greater aperture and greater transparency of eye media, but the extra density of pigment would be much less advantageous because an increase in density is not only accompanied by an increase in signal given by the more effective absorption of the incident light quanta, but also by an increase in 'noise' consequent in the increase in the amount of visual pigment which achieves this extra absorption. The 'noise' will go up as the square root of the density of pigment in the rod and the signal as the fraction of light absorbed. The optimum density of pigment is such that 71·5 % of the light is absorbed (density of 0·55) (Denton & Pirenne) and then the gain over the human would only be × 1·25, not × 3, as simple computation of absorption might suggest. The higher densities found in some deep-sea fish would give an even smaller gain than × 1·25 over the human. The deep-sea fish probably has, however, an advantage over man for, with an eye at a lower temperature and a photosensitive pigment which although similar in shape to that of visual purple is displaced towards the blue, the spontaneous rate of breakdown of visual photosensitive pigment might be expected to be much lower. To have an idea of the order of such an effect we may consider, as Stiles (1948) and Barlow (1957) have done, a simplified picture where the molecules of pigment have thermal energies distributed in accordance with Boltzmann's distribution, when a reduction in temperature from the mammalian temperature to that of a deep-sea fish will make the pigment × 10 more stable, whilst the effect of the shift of maxima towards the blue from 502 to 485 mμ might be expected to be accompanied by a further tenfold increase in stability. Bridges (1956) finds that in a mixture of two extracted visual photosensitive pigments with maxima at 522 and 533 mμ respectively, the first broke down spontaneously in the dark at a rate forty times more slowly than the second (see also Dartnall, 1955).

Thus on the basis of the 'noise' theory allowing for a more stable retina we arrive at a revised estimate of the sensitivity gain of the deep-sea fish eye over the human eye of 60–120 times instead of 15–30 times, giving an extra range of vision of daylight of about 40 m deeper than that calculated above, and putting the maximum depth at which the deep-sea fish can see daylight at about 1150 m.

We have not taken account of the possibility of extra physiological summation in area and time in the retinae of deep-sea fish, but the eyes of deep-sea fish are often small and would not permit of much extra area summation over that found in humans, whilst against physiological summation obtained by the absorption of quanta over larger areas and times must be set the possibility of probability summation between independent retinal units. The argument is, however, not a profitable one to pursue because as the intensity of daylight penetrating the ocean falls with depth, the light given by luminescent creatures becomes more and more predominant and no matter how sensitive the eye, the daylight is small compared with this light and the problem is one of intensity discrimination not one of absolute sensitivity. Our estimate of 1150 m is already down at depths at which this must be true.

We are grateful to Mr N. B. Marshall and Dr W. A. H. Rushton for their advice and help, and to the Director of the National Institute of Oceanography for his kindness in giving permission to carry out experiments aboard R.R.S. *Discovery II*. We also wish to express our thanks to Captain C. A. Hoodless and the crew of R.V. *Sarsia* and to Captain S. S. F. Dalgleish and crew of the R.R.S. *Discovery II* for their enthusiastic co-operation.

SUMMARY

Measurements were made on the intact retinae dissected from freshly caught deep-sea fish. The unbleached retinae of such fish are not rose-pink like the retinae of most coastal fish or purple like the retinae of most freshwater fish, but are golden in colour.

The golden colours are of photosensitive pigments which give retinal absorption curves similar in shape to frog's rhodopsin, but with maxima of absorption displaced on the average about 15 mμ towards the blue end of the spectrum. The names 'chrysopsins' or visual golds are suggested for this group of pigments.

The density of such photosensitive pigments is often very high. Retinal density changes on bleaching of more than 1·0 has been found for several deep-sea fish. These probably correspond to absolute retinal densities of pigment of about 1·3, i.e. the absorption of 95% of blue-green light striking the retina.

The conger and silver freshwater eels have retinae containing similar golden-coloured pigments. These eels begin their lives in the deep ocean and return there when mature to spawn.

The significance of this type of photosensitive pigment in the vision of deep-sea fish is discussed, and an estimate is made of the depths at which deep-sea fish will see daylight.

REFERENCES

BARLOW, H., 1956. Retinal noise and absolute threshold. *J. opt. Soc. Amer.*, Vol. 46, pp. 634–9.

—— 1957. Purkinje Shift and Retinal Noise. *Nature, Lond.*, Vol. 179, pp. 255–6.

BEEBE, W., 1935. *Half Mile Down.* London: John Lane.

BRIDGES, C. D. B., 1956. The visual pigments of the Rainbow trout (*Salmo irideus*). *J. Physiol.*, Vol. 134, pp. 620–9.

CLARKE, G. L., 1936. On the depth at which fish can see. *Ecology*, Vol. 17, pp. 452–6.

CLARKE, G. L. & WERTHEIM, G. K., 1956. Measurements of illumination at great depths and at night in the Atlantic Ocean by means of a new bathyphotometer. *Deep-Sea Res.*, Vol. 3, pp. 189–205.

DARTNALL, H. J. A., 1955. Visual pigments of the bleak (*Alburnus lucidus*). *J. Physiol.*, Vol. 128, pp. 131–56.

DENTON, E. J., 1954. Une novelle méthode pour déterminer la courbe d'absorption du pourpre rétinien. *C.R. Acad. Sci., Paris*, T. 239, pp. 1315–6.

DENTON, E. J. & PIRENNE, M. H., 1954. The absolute sensitivity and functional stability of the human eye. *J. Physiol.*, Vol. 123, pp. 417–42.

DENTON, E. J. & WALKER, M. A., 1957. The visual pigment of the conger eel. (In preparation.)

DENTON, E. J. & WARREN, F. J., 1956. Visual pigments of deep sea fish. *Nature, Lond.*, Vol. 178, p. 1059.

DENTON, E. J. & WYLLIE, J. H., 1955. Study of the photosensitive pigments in the pink and green rods of the frog. *J. Physiol.*, Vol. 127, pp. 81–9.

JERLOV, N. G., 1947–8. Optical studies of ocean waters. *Rep. Swedish deep-sea Exped.*, Vol. 3, Fasc. 1, Physics and Chemistry, pp. 1–59.

KÜHNE, W., 1878. *The Photochemistry of the Retina and on Visual Purple*. (Translated by M. Foster.) London: Macmillan.

LE GRAND, Y., 1948. Lumière et couleurs. *Optique Physiologique*, Vol. 2. Paris. Editions de la Revue d'Optique.

MARSHALL, N. B., 1954. *Aspects of Deep Sea Biology*. London: Hutchinson's.

RUSHTON, W. A. H., 1956. The rhodopsin density in the human rods. *J. Physiol.*, Vol. 134, pp. 30–46.

ST GEORGE, R. C. C., 1952. The interplay of light and heat in bleaching rhodopsin. *J. gen. Physiol.*, Vol. 35, pp. 495–517.

STILES, W. S., 1948. The physical interpretation of the spectral sensitivity curve of the eye. *Transactions of the Optical Convention of the Worshipful Company of Spectacle Makers*, 1948, pp. 97–107. London.

WALD, G., 1945–6. The chemical evolution of vision. *Harvey Lect.*, Ser. 41, pp. 117–60.

WALD, G., 1938–9. The porphyropsin visual system. *J. gen. Physiol.*, Vol. 22, pp. 775–93.

Note added in proof

F. W. Muntz has recently confirmed (*Science*, 1957, Vol. 125, pp. 1142–3) that deep-sea fish have golden-coloured retinae. He has also shown that *Bathylagus wesethi* Bolin has a retinae containing a mixture of two photosensitive pigments.

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STUDIES IN THE GENUS *FUCUS* L.

II. DISTRIBUTION AND ECOLOGY OF FORMS OF *FUCUS DISTICHUS* L. EMEND. POWELL IN BRITAIN AND IRELAND

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The Marine Station, Millport

(With Plates I–IV and Text-figs. 1–5)

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INTRODUCTION

In part I of this series (Powell, 1957) the species *Fucus distichus* L. was re-established and emended to include the following four subspecies: subsp. *distichus*, subsp. *anceps* (Harv. et Ward ex Carruthers) Powell, subsp. *edentatus* (De la Pyl.) Powell, and subsp. *evanescens* (C. Ag.) Powell. Of these subspecies, only *anceps*[1] and *edentatus* have been found in the British Isles. Their distribution and ecology in Britain and Ireland are now described.

[1] Throughout the text subspecific names have to be referred to constantly, and will be cited normally as a straight trinomial, e.g. *F(ucus) distichus anceps*. Forms and other lower categories are indicated in the usual way, e.g. *F. vesiculosus* f. *linearis*. Often, to avoid tedious repetition, it is adequate to refer simply to the final epithet, whether subspecies or form, e.g. *anceps*, or *linearis*, alone. Among names of other organisms there are some in which the genus here automatically indicates a single species (e.g. *Alaria esculenta*, *Chthamalus stellatus*), and the specific name can often be dropped.

It is probable that, until the present century, authentic specimens of any form of *Fucus distichus* L. emend. Powell were known in the British Isles only from Kilkee, Co. Clare, Ireland.[1] There, in 1863, W. H. Harvey and N. B. Ward found and described *F. anceps* (see Powell, 1957, for references). Many of the larger herbaria in Britain contain specimens of '*F. anceps*' from Kilkee, most of the specimens being distributed by E. M. Holmes (*Algae Britannicae Rariores Exsiccatae*, Fasc. X, No. 240). As the data on numerous herbarium specimens examined testify, several other collections were made at Kilkee later in the 19th century, the latest known date being September 1897 (collected by 'E. George').

The first authentic British record of *F. distichus edentatus* is that of Börgesen (1903) for the Shetland Islands (14–16 July 1902). He reported that 'well-developed, typical specimens, agreeing exactly with my Faeröese specimens referred to [*F. inflatus*] f. *edentata* (De la Pyl.) Rosenv., occurred abundantly near Lerwick'.

Batters (1902) and Newton (1931) both list *F. anceps* Harv. & Ward as a distinct species (with Kilkee as the only locality) and both mention, but with some doubt, a record by Traill (1885) of '*F. distichus*'.[2]

I first became interested in *F. distichus* during the summer of 1951 when with Dr J. R. Lewis (Leeds) I carried out an ecological survey of the north coast of Scotland. One of the most interesting findings of the survey was the discovery of *F. distichus anceps* along a limited and very exposed stretch of the north coast of Caithness. This remains the only record of any form of *F. distichus* for the mainland of Britain, and in a preliminary announcement of the find (Powell & Lewis, 1952) Börgesen's nomenclature was followed.

During succeeding years I have had opportunities of taking part in several phycological surveys of various parts of the coast of north and west Britain and Ireland, as follows: Fair Isle and part of the Mainland of Shetland in 1952, and parts of the west of Ireland in 1953, in each case with colleagues Dr E. M. Burrows (Liverpool), Dr E. Conway (Glasgow) and Dr S. M. Lodge (Liverpool); and the island of Lewis-Harris (Outer Hebrides) in 1954. These expeditions have yielded further information about the distribution

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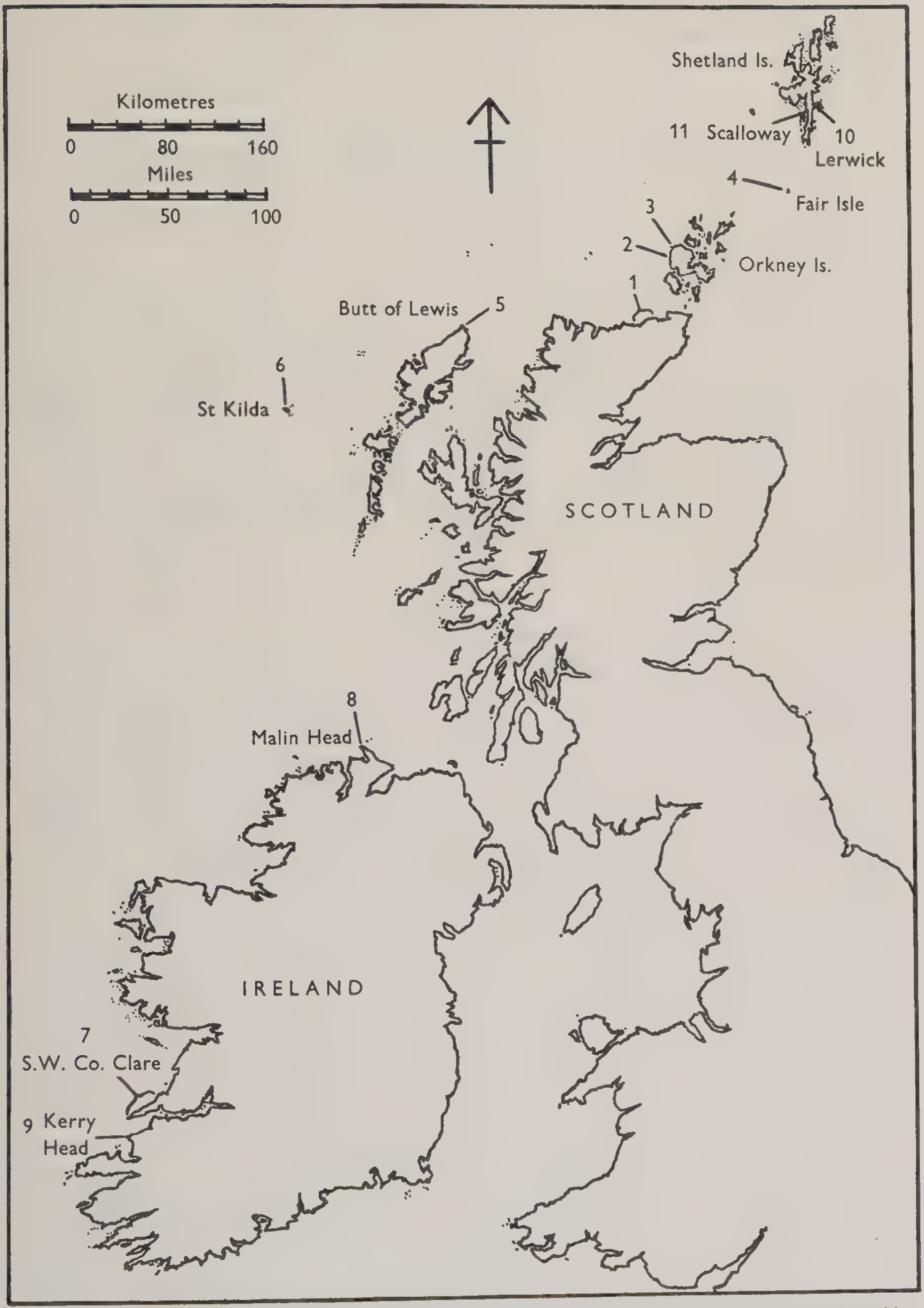
[1] All other early British records of fucoids that might possibly have referred to this species (e.g. the '*F. distichus*', '*F. inflatus*' and '*F. linearis*' of various authors) have been carefully considered, and almost certainly all refer to forms of either *F. ceranoides* L. or *F. vesiculosus* L. Thus '*F. distichus*' of Lightfoot (1777, p. 912) was probably a very narrow form of *F. ceranoides*; and '*F. inflatus*' Lightfoot (1777, p. 910) and '*F. linearis*' Hudson (1762, p. 467) were forms of *F. vesiculosus.*

[2] Traill (1885, p. 11) records '115. *Fucus distichus.* Cast ashore at Port-Seton (J. R. Henderson, 1882). Only one specimen found. Identified by E. M. Holmes.' I have made unsuccessful efforts to trace this specimen; it is *not* in Herb. Traill (University of Edinburgh), Herb. Holmes, Herb. Batters or General Algal Herb. (British Museum), or Herb. Kew. The specimen probably cannot be traced now and it would seem best to disregard this very doubtful record.


Kilometres
0
80
160
Miles
0
50
100
Shetland Is.
11 Scalloway
10
Lerwick
4
Fair Isle
3
2
Orkney Is.
1
Butt of Lewis
5
6
St Kilda
SCOTLAND
8
Malin Head
IRELAND
7
S.W. Co. Clare
9 Kerry
Head

Text-fig. 1. Map to show localities (numbered 1–11) at which *Fucus distichus* has been found in Britain and Ireland. Subsp. *anceps* has been found at localities 1–9, and subsp. *edentatus* at localities 4, 10 and 11.

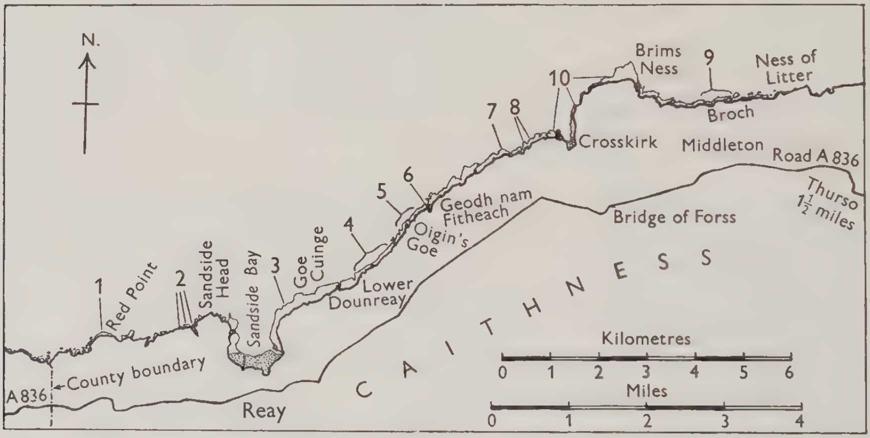
and ecology of forms of *F. distichus*; also, a number of additional valid British records have come to my notice from various sources and are discussed below.

FUCUS DISTICHUS L. EMEND. POWELL, SUBSP. *ANCEPS* (HARV. ET WARD EX CARRUTHERS) POWELL (Pl. I)

DISTRIBUTION

Scotland (see Text-figs. 1, 2)

CAITHNESS. Found, intermittently, only along a limited stretch (about 12 miles) of the north coast, from a point about 3 miles west of Holborn Head (near Thurso) westwards to near the county boundary with Sutherland (July–September 1951). It is possible that the range may extend slightly farther, both east and west, on reefs that are difficult to reach. The principal reefs on which *anceps* was found in the 1951 survey are shown in Text-fig. 2, sites 1–9; more recently (August 1954 and June 1956) Dr E. Conway (accompanied by my wife, Grace Powell, in 1956) has found the plant also at the three sites marked 10.


N.
Brims Ness
9
Ness of Litter
10
7
8
Broch
Crosskirk
Middleton
Road A 836
Thurso 1½ miles
6
5
Geodh nam Fitheach
Oigin's Goe
Bridge of Forss
4
Goe Cuinge
3
Sandside Bay
Sandside Head
2
1
Red Point
Lower Dounreay
CAITHNESS
Kilometres
0 1 2 3 4 5 6
Miles
0 1 2 3 4
County boundary
A836
Reay

Text-fig. 2. Map of part of the north coast of Caithness. The principal sites at which subsp. *anceps* has been found are numbered 1–10 (see text).

ORKNEY ISLANDS. Recorded only from three sites, all on the west coast of the Mainland of Orkney. The plants from sites 1 and 2 (Text-fig. 1, locality 2), situated just south and just north of Bay of Skaill, were collected by James Sinclair (Orkney) in 1938 and 1939, respectively. I first heard of them through correspondence with Dr Søren Lund (Charlottenlund, Denmark), and later Mr Sinclair kindly allowed me to examine and identify his herbarium specimens and to refer to his records here. Details are (1) Hole o'Rowe, Sandwick, Orkney (59° 3′ 4″ N., 3° 21′ 21″ W.), 16 October 1938, common in one spot on an exposed ledge of rock, material all sterile, sheet no. 445 in Herb. J. Sinclair; (2) exposed coast below Garson, Northdyke, Sandwick, Orkney (59° 3′ 40″ N., 3° 21′ W.), 7 May 1939, common on flat rocks, all five herbarium specimens fertile, sheet no. 511 in Herb. J. Sinclair.

For the third Orkney record I am indebted to Dr A. J. Southward (Plymouth), who in May 1953 found one small patch of *anceps* on an exposed reef near Brough Head, north-west Orkney Mainland (Text-fig. 1, locality 3; approximate position 59° 8′ 16″ N., 3° 19′ 39″ W.); the area occupied by the plant was several metres long by 0·5 m wide, on the upper shore among *Chthamalus stellatus* (Poli); material fertile.

FAIR ISLE. Found only at two sites in June–July 1952. At North Gavel, a very exposed site (59° 32′ 28″ N., 1° 35′ 49·5″ W.), *anceps* was fairly common on high-level ledges and rock steps (see Burrows, Conway, Lodge & Powell, 1954). At a second site, occasional scattered plants were found on extremely exposed reefs (59° 30′ 53″ N., 1° 39′ 7·5″ W.) immediately to the west of the South Lighthouse.

OUTER HEBRIDES. *Island of Lewis-Harris.* Cotton (1912, pp. 23–6), in relating the zonation of fucoids in parts of the west of Ireland to the rest of Britain,[1] and to other places in Europe, mentions (p. 23) 'An additional species, *F. inflatus*, was recorded by Börgesen for the Shetland Isles and Mr E. M. Holmes has lately received a specimen of this boreal species from Lewis, so possibly it is a regular constituent of the association in the north of Scotland.' The specimens from Lewis referred to by Cotton are now located in Herb. Kew, and detailed examination has proved that the plants are true *F. distichus* subsp. *anceps*. There are three specimens in Herb. Kew, on separate sheets, as follows: (*a*) a single narrow specimen, labelled '*Fucus inflatus* var. *distichus*, Butt of Lewis, N.B. Coll. W. J. Gibson. April 1909. Comm. E. M. Holmes.' Another label on the sheet indicates that the plant was actually named by F. Börgesen; and attached to the sheet is a letter from W. J. Gibson ('The Nicholson Institute, Stornoway') to E. M. Holmes dated 21 April 1909, stating that the material was collected 'last week' (i.e. about 14 April 1909). Specimen (*b*) is a duplicate of (*a*), labelled similarly, and mounted singly on a separate sheet. (*c*) On another sheet (beside three specimens of authentic '*Fucus anceps*, Kilkee') is mounted one plant labelled '*Fucus distichus*? Stornoway, N.B. Comm. E. M. H. May '09.' I am inclined to think that this is simply another duplicate Gibson specimen from 'Butt of Lewis' that Holmes sent on to someone else (or possibly to Kew) in May 1909; certainly it could not really have been collected in the sheltered environs of 'Stornoway'. All three specimens are fertile.

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[1] Curiously, however, Cotton omits to mention in this general discussion Harvey's important record of '*F. anceps*' from Kilkee.

In July–August 1954 I was able to investigate the extreme northern tip of Lewis, but only the accessible parts of the shore north of a line running east to west from just south of Aird Dell on the west coast to just north of Port Skegirsta on the east coast. I found subsp. *anceps* only at two sites (close together; 58° 30′ 3″ N., 6° 13′ 19″ W.) on very exposed reefs, facing E.N.E., at the foot of the headland named Buaile na Faing, which is about 2 miles south-east of the Butt of Lewis. The littoral area of the actual headland and small islets comprising the true Butt of Lewis is precipitous and inaccessible without a rope or a boat and could not be investigated in 1954. Possibly the location 'Butt of Lewis' cited by W. J. Gibson is used in a broad sense, to include some few miles of the northern tip of Lewis.

St Kilda. Algae from the 1952 marine biological expedition to St Kilda (Gauld, Bagenal & Connell, 1953) were identified by me. *F. distichus* subsp. *anceps* (listed as *F. inflatus* f. *distichus*) was found in small quantity (a few plants) at one site only—at the top of the *Balanus balanoides* (L.) belt, on rather steeply sloping rocks at the head of Glen Bay, on the north side of Hirta (57° 49′ 16″ N., 8° 35′ 50″ W.). T. B. Bagenal (Millport) revisited the Glen Bay site on 14 July 1956 and again found *anceps*, in late fertile condition. He reports that the fucoid had greatly increased in amount since 1952, forming a belt up to 1 m wide at the head of the bay, and had spread (although not continuously) along about 100 m of shore altogether.

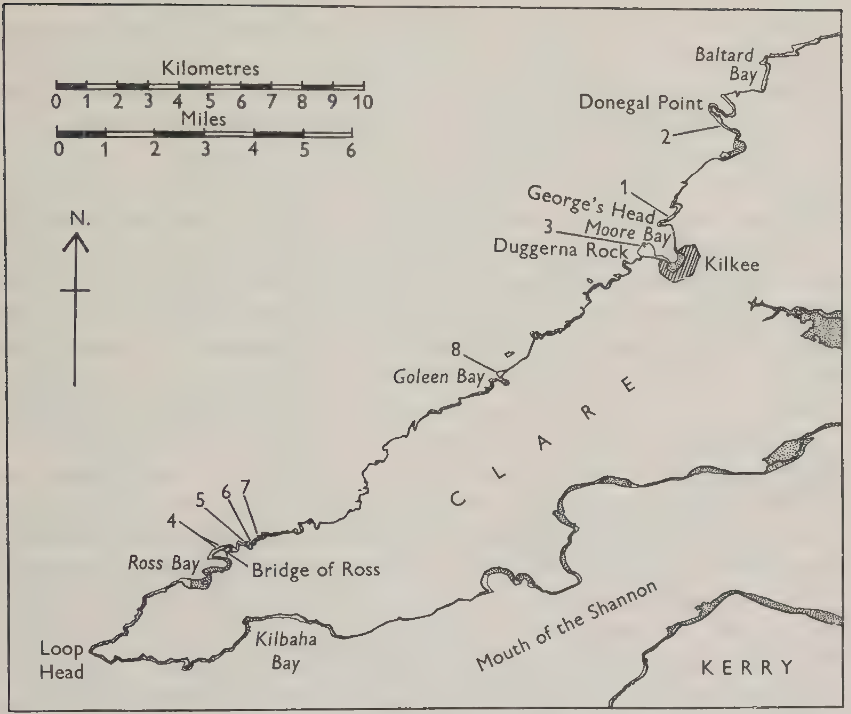
Ireland (see Text-figs. 1, 3)

KILKEE DISTRICT, Co. Clare. (Text-fig. 3.) In July 1953 *anceps* was found at the precise site (Duggerna Rock) at Kilkee where W. H. Harvey first recorded it 90 years before. During the 1953 survey the plant was found also on exposed rocks at several other localities in south-west Co. Clare, both north and south of Kilkee, between Donegal Point and Ross Bay, the exact sites being: (1) George's Head (north side, well inshore, on reefs facing N.N.W.); (2) Donegal Point (midway along south-west flank; the Point itself is a sheer 100 ft. cliff); (3) Duggerna Rock, Kilkee (and on two reefs immediately adjacent to and west of the Rock); (4)–(7) at four separate exposed sites, all within 1 mile of coast, just north of Ross Bay (i.e. from near Bridge of Ross, north-east for about a mile); (8) Goleen Bay (exposed reefs at northern entrance to Bay).

Also in 1953, J. R. Lewis surveyed other parts of the coast of Ireland and found *anceps* at two additional, isolated, exposed sites (Text-fig. 1, localities 8 and 9) as follows:

MALIN HEAD (the northernmost point of Co. Donegal), 4 July 1953, on reefs facing N.N.W. about 250 yards west of Malin Tower (55° 22′ 53″ N., 7° 22′ 56″ W.).

KERRY HEAD, Co. Kerry (52° 25′ 9″ N., 9° 56′ 44″ W.), 6 June 1953. This is the most southerly record of any form of *F. distichus* in Europe.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N.
Baltard Bay
Donegal Point
2
1
George's Head
3
Moore Bay
Duggerna Rock
Kilkee
8
Goleen Bay
C L A R E
5 6 7
4
Ross Bay
Bridge of Ross
Loop Head
Kilbaha Bay
Mouth of the Shannon
KERRY

Text-fig. 3. Map of south-west part of Co. Clare. The sites at which subsp. *anceps* has been found are numbered 1–8 (see text).

DISTINCTION FROM OTHER FUCOIDS

The distribution and ecology of *F. distichus anceps* is most conveniently considered in relation to the other fucoids found in Britain. On sheltered rocky shores, as is well known, the zonation of the littoral Fucaceae association follows a fairly standard pattern. The species occur in more or less well-defined belts in the following order from high-water level downwards: *Pelvetia canaliculata* (L.) Dec'ne et Thur., *Fucus spiralis* L., *Ascophyllum nodosum* (L.) Le Jol./*Fucus vesiculosus* L., *F. serratus* L. With increasing exposure to wave-action some of these dominants are replaced either by other algal species or by animals, but some persist usually in a modified and often reduced form. *Ascophyllum* is usually the first of the series to be eliminated by increasing exposure, followed by the normal bladdered form of *Fucus vesiculosus*, and then *F. serratus*; thus the fucoid zonation on fairly exposed and moderately steep shores is often as follows: *Pelvetia*, reduced plants of *Fucus spiralis*, *F. vesiculosus* f. *linearis* (Huds.) Powell (=f. *evesiculosus* auctt.). With further increase in exposure, or on steeper shores, the normal form of

F. spiralis is usually next lost from the flora, followed by *Pelvetia* and finally by *Fucus vesiculosus* f. *linearis*. Very steep, fully exposed shores usually have no fucoids at all. However, on some of our exposed coasts, *F. spiralis* is represented by the small form known as *F. spiralis* f. *nana* (Stackh.) Batt., which, where present, usually persists into conditions of even greater general exposure than tolerated by *F. vesiculosus* f. *linearis*.

The usual position of *F. distichus anceps* in this general scheme is that it first appears in the flora only under conditions of very considerable exposure to wave-action and oceanic swell, forming a narrow belt between the belts of *F. spiralis* f. *nana* (above) and *F. vesiculosus* f. *linearis* (below), and mingling with these fucoids to varying extent. In the Scottish localities *anceps* then persists into more severe exposure conditions than *nana*, but in the Kilkee district of Ireland *nana* withstands rather more exposure than *anceps*, suggesting that *anceps* is less tolerant of adverse conditions near the southern limit of its range.

Thus, in Britain, three species of *Fucus* may persist on exposed coasts; and, with increasing exposure, all three species show reduction along similar lines, all becoming both shorter and narrower. Of the three forms, *F. vesiculosus* f. *linearis* is the most variable and in certain localities and circumstances can closely resemble either *F. distichus anceps* or (to a lesser extent) *F. spiralis* f. *nana*. Indeed, all three forms could well be (and have been) confused by investigators not familiar with all three species, and means of distinguishing these forms both in the field and in the laboratory will now be described.

F. vesiculosus f. *linearis* (Pl. III, fig. 2, Pl. IV, fig. 1; Cotton, 1912, Pl. I; frontispiece, *Rev. Algologique*, T. 5, fasc. 3–4, 1931; Harvey, 1950, for photograph of plants on Lundy) is extremely variable in form and in conditions of considerable exposure may be reduced to rather erect stunted tufts only a few inches in length and with narrow fronds (but never so narrow as fronds of *anceps* growing at the same site). Fundamentally *linearis* is distinguished from *anceps* by the fact that it is unisexual and dioecious, while *anceps* is invariably hermaphrodite (it should be noted, however, that very old conceptacles of *anceps* may have released all antheridia and may then show a few remaining oogonia only). Also, the midrib in the younger branches of *linearis* is always very distinct and the lateral alae clearly differentiated (even in very narrow thalli), whereas in *anceps* the midrib and the lateral wings are not very sharply differentiated, the distal branches often being practically oval in section (see Plates I, III and IV). These two characters serve to distinguish the two forms in any circumstances and, in addition, *anceps* usually has a variable (small) number of small caecostomata (not present in either *linearis* or *nana*).

At certain exposed places in south-west England and Wales (e.g. the very exposed islands of Lundy and Skokholm) and in the west of Ireland, *linearis* may develop long, narrow, pointed receptacles, very reminiscent of *anceps* (see

Pl. IV, fig. 1). Gillham (1954, p. 217) records '*Fucus inflatus* L.? (*F. anceps* Harv. et Ward)' for Skokholm and Grassholm, but comments that 'it is possible that the plants are merely an extreme rough-water form of *F. vesiculosus*'. In view of the distribution of *anceps* outlined in the present paper, it is practically certain that the plants in question were merely small and narrow *F. vesiculosus* f. *linearis*;[1] Dr Gillham now agrees with this view.

F. spiralis f. *nana* (Pl. IV, fig. 2) varies in size from a few to *ca.* 10 cm in length and has narrow, strap-like thalli, either unbranched or with few dichotomies, and with very distinct midrib and lateral wings. The receptacles are usually small, terminal, globular, and have the sterile rim of tissue characteristic of this species; conceptacles hermaphrodite. The prominent midrib and the shape of receptacle readily distinguish this form from *anceps*; and the hermaphrodite conceptacles, and sterile rim to the receptacles, distinguish it from the most reduced forms of *linearis*.

It is usual for plants of *nana* to have all the apices fertile at the same time; this is a common feature also in *anceps*, and to a lesser extent in *linearis*, especially when the last two forms are growing under the most rigorous conditions that even they can tolerate. The development of this feature in the least favourable environmental circumstances is doubtless of value for the survival of the species.

ECOLOGY AT PARTICULAR SITES

Scotland

Caithness

The 1951 survey included an examination of many sites on the north, north-east and north-west coasts of Scotland (Sutherland and Caithness), but *F. distichus anceps* was found only on very exposed reefs at the sites indicated in Text-fig. 2. Geologically the reefs of the north coast of Caithness are formed of the remarkable Caithness Flagstones (a group of Middle Old Red Standstone), and form virtually unbroken and often very extensive sheets of rock, sloping either very gently or more or less steeply, sometimes from well above effective high-water level down into deep water. More usually, however, the reefs occur in a series one behind the other and more or less parallel with the coast-line, all dipping seawards and with the outermost being very much more exposed than the inner reefs which are comparatively sheltered.

Most of the reefs face north-west or north and, since they dip down directly into deep water and are not protected by offshore reefs or islands, are fully exposed both to oceanic swell and to all the wave-action produced by more local winds. Even during periods of local calm weather these reefs are constantly subject to oceanic swell which breaks as it travels inshore and on the gently inclined reefs sweeps upshore at great speed and may inundate all the lower half of the effective littoral zone even at low tide. As a result, there is a considerable increase in the effective height of the littoral zone, and the littoral plants and animals are able to occupy wider (vertical) zones without suffering undue desiccation. Also the communities of plants and animals present, in the upper littoral especially, are usually much more open than in sheltered localities. The general ecology of the dominant plants and animals found on these remarkable reefs has been described and illustrated by Lewis (1954).

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[1] This belief was strengthened when the author had an opportunity to examine some exposed parts of the shore of Skokholm during September 1956. *F. vesiculosus* f. *linearis* was common but no plants of *F. distichus anceps* were found.

In bays and other sheltered sites between the extensive Flagstone reefs, all the usual fucoids of sheltered British coasts were found. With increasing exposure, some of these were eliminated in the usual sequence and *F. vesiculosus* f. *linearis* became common in the upper part of the mid-littoral zone. On the upper shore, *Pelvetia canaliculata* and *Fucus spiralis* became represented by smaller plants, but *Pelvetia* was soon lost from the open reefs and *Fucus spiralis* persisted only as the small f. *nana*.

Moderately exposed (or very gently sloping) reefs characteristically showed the following zonation of dominant species from low-tide level upwards. *Sublittoral fringe*: dominated by *Alaria esculenta* (L.) Grev. *Mid-littoral zone*: first a broad belt of *Himanthalia elongata* (L.) S. F. Gray, merging upwards into a belt of *Rhodymenia palmata* (L.) Grev.; then a belt of *Gigartina stellata* (Stackh.) Batt./*Balanus balanoides*/*Mytilus edulis* L.; next a belt of *Fucus vesiculosus* f. *linearis*/*Balanus*/*Mytilus*, with the *Balanus* extending to a slightly higher level than the *Fucus*. *Supralittoral fringe*: dominated principally by a wide belt of *Porphyra umbilicalis* (L.) Kütz. (f. *umbilicalis*), with a weft of less conspicuous blue-green algae growing beneath the *Porphyra* plants and also extending to higher levels than *Porphyra*. Frequently patches of *Blidingia minima* (Näg.) Kylin occurred in the lower part of the *Porphyra* belt and extended into the upper part of the mid-littoral zone. Thus, *Fucus vesiculosus* f. *linearis* was often the only fucoid found on such a gently sloping reef.

On more exposed reefs (either those farther out or those with steeper slope) a belt of *F. spiralis* f. *nana* appeared in the lower half of the supralittoral *Porphyra* belt. (In some few instances it was possible to trace a full series of intermediate forms of *Fucus spiralis* from the typical form in shelter through to f. *nana* with more exposure, but more usually f. *nana* occurred as locally isolated populations on the more exposed reefs.) With still greater exposure (on the steeper reefs), *F. distichus anceps* appeared and formed a distinct belt, *ca.* 15–45 cm vertical range, at the level of the general upper limit of *Balanus balanoides*—i.e. between the belts of *Fucus spiralis* f. *nana* (above) and *F. vesiculosus* f. *linearis* (below) where these were also present. With still greater exposure, *F. vesiculosus* f. *linearis* and *F. spiralis* f. *nana* were in turn lost from the flora, leaving *F. distichus anceps* as the sole remaining fucoid on many of the steeper (most exposed) reefs. Still steeper reefs and vertical walls of rock had no fucoids at all; such sites were dominated principally by a high-level belt of *Porphyra*, mid-littoral *Balanus* and *Mytilus*, and *Alaria* on the lower shore.

On the more exposed reefs *Fucus distichus anceps* was often locally abundant (see Pl. I, fig. 1). The smallest plants were those growing at the most exposed sites; at rather less exposed sites the plants were larger and the largest of all were intermediate in size and habit between subsp. *anceps* and subsp. *edentatus* (see Pl. II, fig. 1). However, such large plants have been found at only a very few restricted sites where forms of *F. vesiculosus* and *F. spiralis* were less dominant than was usual with increasing shelter.

The uplifted, open communities of algae developed on the exposed reefs became confined to ever narrower and more exclusive belts on the more sheltered inshore reefs. It is possible that competition from forms of more successful fucoids (*F. spiralis* and *F. vesiculosus*) prevents the establishment (to any great extent) of intermediate forms of *F. distichus* on reefs with intermediate exposure conditions. Or, stated otherwise, *F. distichus anceps* is most abundant on the more exposed reefs probably because generally it is only on these reefs that a suitable 'ecological niche' is regularly available that it can successfully occupy; the equivalent (but narrower) zone on more sheltered reefs becomes increasingly occupied by forms of *F. spiralis* and *F. vesiculosus*.

These general conclusions are based on an examination of many reefs. However, along the 12–15 miles of shoreline in question local variations from the zonation of Fucaceae outlined above were occasionally found. Sometimes these variations could be explained as the result of greater or less exposure very locally, caused by the particular configuration of the reef(s) in question (aspect, angle of slope, and relation to other nearby reefs)—e.g. the presence of a patch of *F. spiralis* f. *nana* above belts of *Pelvetia* and typical *Fucus spiralis* near the sheltered head of one of the inner reefs could be explained in terms of wave-action on the (more exposed) reef in front; waves and swell sweep up the outer reef and some splash dashes over and wets the small area of rock at a high level on the reef behind, just sufficiently for *F. spiralis* f. *nana* to develop there. Observations such as this serve to emphasize that, although *nana* and *anceps* were usually confined to the reefs most exposed to wave-action, these particular forms occur at a high level in a much extended littoral zone, and do not suffer most of the violence of swell and wave-action experienced by plants such as *Alaria* on the lower shore; on the other hand, the upper zones benefit from the almost constant spray of waves breaking lower down the shore.

On several very exposed reefs which otherwise lacked fucoids, patches of *anceps* were present only on the ends of the reefs most affected by spray from breaking waves; again it is probably the spray that is beneficial to this high-level form rather than any possible direct effect of wave-action.

Occasionally, both *linearis* and *nana* were absent from reefs where they were expected to occur. Possibly these reefs are stripped of such populations during exceptionally rough weather.

Fair Isle

At the North Gavel site on Fair Isle (see Burrows *et al.*, 1954) the survey party made a fairly accurately levelled shore transect, to compare the vertical zonation of dominant algae with that developed at the sheltered North Haven not far away. The transects and vertical zonations are given in detail in Burrows *et al.* (1954), which also includes photographs (Pl. 15) of the upper and lower parts of the exposed North Gavel site. The belts of dominant algae present were very similar to those described above for Caithness, but the configuration of the shore was interestingly different. Thus the lower shore, covered with *Alaria esculenta*, consisted of a gently sloping platform, which below low-water level sloped very steeply into deep water; the whole of the *Alaria* platform was constantly washed by swell even on calm days. Above the wide belt of *Alaria* the shore consisted of a series of steep sloping walls and rather flat narrow ledges, backed finally by cliffs. On the line of the transect, the steep mid-littoral faces bore little else but scattered tufts of *Gigartina stellata*, but on less steep mid-littoral slopes nearby, *Balanus balanoides*, *Mytilus edulis* and *Patella aspera* Lamarck were common, together with *Rhodymenia palmata*, *Gigartina stellata*, *Acrosiphonia centralis* (Lyngb.) Kjellm., *Scytosiphon lomentaria* (Lyngb.) Endl., *Callithamnion arbuscula* (Dillw.) Lyngb. and *Ceramium acanthonotum* Carm. ex Harv.; the smaller of these mid-littoral algae were mostly attached to the animals (as often they were in Caithness). Higher still, *Fucus distichus anceps* formed a narrow belt (*ca.* 0·6 m in vertical extent) on rock steps, and among and above it *F. spiralis* f. *nana* was particularly well developed, dominating rock steps through a vertical range of about 1·5 m. A belt of mixed *Porphyra umbilicalis* and *Blidingia minima* extended upshore for 1·5–2·1 m (vertical) on rather steep rock faces above *Fucus spiralis* f. *nana*.

The range of spring tides quoted in *The Admiralty Tide Tables* (1956) for Fair Isle is 1·74 m, but the vertical height of rather steep shore occupied by algae at the exposed North Gavel site was up to 6·1 m (from low-water level to the top of the

Blidingia minima zone). As shown in Burrows *et al.* (1954), the top of the *Alaria* belt corresponded approximately to observed high-water of a neap tide on a calm day, but even on such a day swell broke continuously at this site, and at high-water repeatedly flooded a zone extending upwards for 1·2–1·5 m above the theoretical high-water level (up to the lowest *anceps* plants), and splash from these waves wetted plants growing up to 2·4–2·7 m above high-water level (including all of the *anceps* belt and most of the main *nana* belt). Throughout most of the year the amount of wave-action experienced at this site will be very much greater, and it may be supposed that the high-level belts of reduced fucoids are frequently within the zone flooded by breaking waves, but probably are not often subjected to the maximum violence of waves breaking directly on them. The survival of belts of algae at these very high levels depends upon their being frequently wetted by swell, waves and splash, or else moistened by mist or rain; and the North Gavel site (facing north-east) is also favoured by shade cast by the cliff behind. Some small plants of *nana* were found, in complete shade, in a small gulley as much as 7·5 m above the main *nana* belt on the open ledges below; a trickle of fresh water kept the plants in the shaded gulley moist.

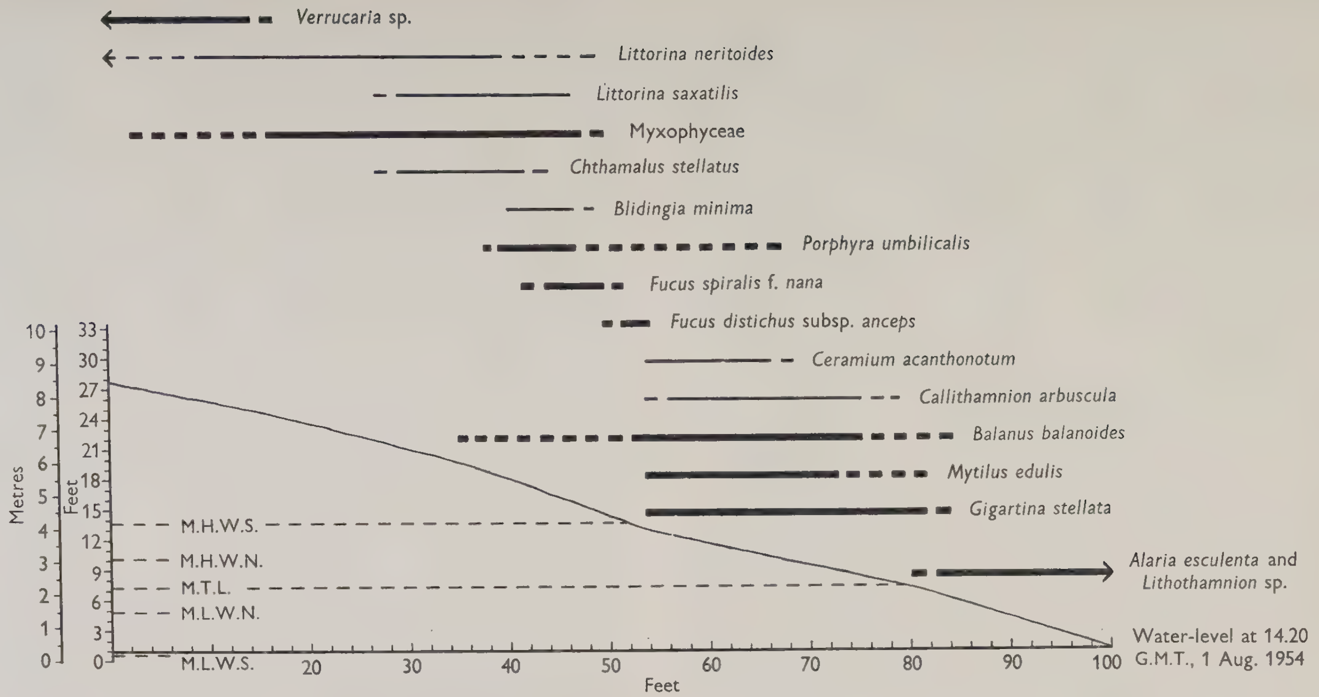
On the whole the plants of *anceps* were smaller than those found on the Caithness reefs and this is probably the result of more rigorous conditions at the North Gavel site, including relatively greater uplift of the whole belt as a result of the unusual (stepped) local rock configuration.

In a shallow high-level pool in the *anceps* belt at North Gavel, two plants of *Fucus distichus* were found which were distinctly narrower than the subsp. *anceps* of open rock and had much shorter receptacles; this narrow pool-plant was in fact intermediate in form between subsp. *anceps* and typical *F. distichus* (subsp. *distichus*) (see Powell, 1957).

At the second site on Fair Isle, a few patches only of *anceps* were found, on very exposed gently sloping reefs facing south-west. Neither *F. spiralis* f. *nana* nor *F. vesiculosus* f. *linearis* was found at or near this site and, as one moved toward more sheltered sites nearby, only one plant of *linearis* was observed. On broad flat reefs a short distance to the north, however, all the usual fucoids of shelter were found. *F. distichus anceps* seems to be only precariously established at this site, and was not found anywhere else on Fair Isle (much of the coast is precipitous and inaccessible); but, at Furse, some *F. spiralis* f. *nana* occurred on the upper shore and, passing into shelter nearby, a whole series of intermediate forms through to the typical form of *F. spiralis* was found.

Island of Lewis-Harris

The site at which *anceps* was found on Lewis in 1954 is accessible from land only by climbing down a 20 m cliff with the aid of a rope. Both the cliff and the shore below are composed of Lewisian Gneiss. The configuration of the shore was very similar to parts of Caithness in that the whole of the littoral zone consisted of a single reef of rather gentle slope for the most part (average *ca.* 15°), with a remarkably smooth and unbroken surface, and dipping directly down into deep water. The whole formed a small natural amphitheatre, limited behind and at each end by steep cliffs. In spite of difficulties, a profile of the shore was made and the zonation of the dominant species measured (Text-fig. 4). The theoretical range of spring tides at this site is *ca.* 4 m, but the coast is very exposed and subject to heavy swell, even though it lies on the east side of the Butt of Lewis. However, it will be seen from Text-fig. 4 that the zones of algae were not uplifted to quite the same extent (in relation to theoretical mean tide levels) as they were at North Gavel, Fair Isle. Thus at the Lewis site the belt of *Alaria* was uplifted only to about theoretical M.T.L., compared with M.H.W.N. (theoretical level) at North Gavel.



Text-fig. 4. Transect at Buaile na Faing headland, Island of Lewis, 1 August 1954. The belts occupied by the dominant species are shown by thick continuous lines, while the zonation of some of the less common species is shown by thinner unbroken lines; in each case the broken lines indicate decreasing frequency of occurrence. The vertical heights of belts were measured from a datum corresponding to the observed level of the sea at 14.20 G.M.T. (approximate predicted time of low water at Stornoway); on the vertical scale, this datum has been made to correspond to the height for low water predicted for Stornoway that day (1·0 ft.). The mean tidal heights indicated against the vertical scale are those given in *The Admiralty Tide Tables* (1956) for Stornoway—a sheltered harbour some 25 miles south of the transect site.

Fucus distichus anceps again occupied a distinct belt (*ca.* 1·0 m broad and *ca.* 0·5 m vertical range) astride the general upper limit of *Balanus balanoides*, mixing with *Gigartina* below and *Fucus spiralis* f. *nana* and *Porphyra* above. The *anceps* belt extended horizontally rather intermittently along about 30 m of this reef, and occurred also on an adjacent vertical wall of rock (at south end of reef) and on a rather flat ledge above it. *F. spiralis* f. *nana* occurred above *anceps* on the open reef in rather small quantity, but was very well developed on high-level flat ledges above the wall just mentioned. The high-level belts of *Porphyra* and Myxophyceae were well developed. *Balanus balanoides* continued sparsely in crevices up to the top of the *Porphyra* belt and even higher. *Chthamalus stellatus* occurred in small numbers in the upper half of the *Porphyra* belt and in crevices higher upshore.

Thus the zonation of the dominant species present was remarkably similar at these three widely separated Scottish *anceps* localities, and reports received with the St Kilda and Orkney specimens indicate that the pattern of zonation was also basically similar at those *anceps* sites. There is also a marked similarity in the over-all topography of all the *anceps* reefs in that all slope more or less evenly down into deep water, without offshore islands or reefs for protection. All sites are subjected to considerable oceanic swell and wave-action, which sweeps well upshore and results in a marked uplift of the zones of dominant species, so that *anceps* and *nana* usually occupy levels well above the *theoretical* level of M.H.W.S., but probably not far from the effective mean high-water level. Because of the constant surge and wave-action, the upper levels of these shores are much affected by splash and spray, which probably largely accounts for the development of good high-level belts of *Porphyra umbilicalis* and Myxophyceae that presumably persist throughout the year.

Ireland

During the 1953 survey several shores were examined between the limits of Clifden (Connemara) and Loop Head (Co. Clare), but *Fucus distichus anceps* was found only at the eight sites named on p. 668, all within a stretch of about 16 miles of coast in south-west Co. Clare. Most of these *anceps* sites face north-west and all are exposed to Atlantic swell. Geologically the sites consist of Millstone Grit and Flagstone Series. With the exception of the Duggerna Rock site (see below), all the other localities were essentially similar to the various Scottish sites in shore configuration—i.e. the shore consisted of remarkably smooth unbroken reefs sloping fairly evenly and steeply down into deep water. The constant swell and wave-action again resulted in a marked increase in the effective height of the littoral zone at most of the sites, but this was not so immediately apparent as in north Scotland because in Ireland the high-level belt of *Porphyra umbilicalis* was often either much reduced or absent in July; however, the belt of *Chthamalus stellatus*, the dominant barnacle on these Irish reefs, was uplifted to a considerable extent at some of the steeper sites (cf. Lewis, 1955).

One of the best sites for *anceps* occurred on the north flank of St George's Head, about 1 mile north of Kilkee (site 1 in Text-fig. 3) and, since the zonation of dominant species there was typical of the zonation at a number of the other Irish sites, the principal physical and ecological features of this reef will be described first.

St George's Head

The site lies at the foot of a broken cliff and was reached with some difficulty. The reef faces N.N.W., slopes smoothly down into deep water (angle of slope estimated to be 10–15°), and was much affected by swell and waves which, coming in from the south-west, mostly ran along the reef as well as slightly up it; the greatest violence of wave-action occurred at the western end of the reef and it was here that the uplift of zones was greatest. Farther inshore (in a north-east direction) the severity of wave-action was less and the reef also becomes less steep. At the most exposed (south-west) end of the reef the upper shore was dominated by *Chthamalus stellatus* and a quite well developed (for Ireland) high-level belt of *Porphyra umbilicalis*; an excellent belt of *Fucus spiralis* f. *nana* occurred in the lower part of the *Porphyra* belt. A few yards farther into 'shelter' a fine belt of *Fucus distichus anceps* came in, and mixed with *F. spiralis* f. *nana* above and *Gigartina stellata*/*Chthamalus*/*Mytilus edulis* below. *Ceramium acanthonotum* occurred on the higher *Mytilus*, and *Callithamnion arbuscula* on *Chthamalus* and rock slightly lower. The lower shore was dominated by *Alaria esculenta*, *Lithothamnion* sp. and frequent stunted *Corallina officinalis* L. Farther into shelter, *Fucus vesiculosus* f. *linearis* first appeared at about the upper limit of *Gigartina*, and gradually became dominant on the inner part of the reef where, as the whole littoral decreased in vertical extent, it soon entirely replaced *Fucus distichus anceps*—one of the best examples of this phenomenon seen anywhere. *Gigartina* also disappeared from the upper mid-littoral zone soon after the loss of *anceps*, as *linearis* came to dominate this level. Unfortunately, it was not possible to level accurately the zones of dominant species at this site, but uplift of the upper zones of algae was not nearly so marked as on some of the Caithness reefs, perhaps because of the oblique angle at which the prevailing swell hits the reef and runs along it rather than directly up it. The presence of a good high-level belt of *Porphyra* on the part of the reef most affected by spray from breaking waves was probably also a result of the relatively small amount of uplift of zones compared with some of the other Irish reefs described below; and, probably for the same reason, the *anceps* plants were rather larger than were found at some of the other Irish sites.

Donegal Point

Most of the littoral zone of this headland is precipitous and inaccessible, and *anceps* was found only at one very restricted site about half-way along the south-west flank (site 2 in Text-fig. 3). The reef was rather steep (*ca.* 20°), fully exposed to violent swell, and dominated mainly by *Chthamalus* (with some few stunted algae) on the middle and upper shore, and by *Alaria* on the lower shore. Small and frequent plants of *Fucus spiralis* f. *nana* formed a fairly well-defined belt below a relatively narrow belt of *Porphyra*. The mid-littoral zone was dominated mainly by *Chthamalus* and *Mytilus*, but some few stunted, narrow plants of *Fucus vesiculosus* f. *linearis* were also present, and occasional tufts of *Gigartina* lower down. Several small patches of *Fucus distichus anceps* occurred, situated mostly between the very open 'belts' of *nana* and *linearis*; the individual plants of *anceps* were very small, mostly vegetative, and conspicuously yellow in colour. Farther along this difficult reef (north-west, towards the point) *linearis* and *anceps* were soon absent from the flora, but *nana* persisted on to steeper and even more wave-beaten reefs.

Duggerna Rock, Kilkee (Text-fig. 3, site 3)

This type-locality for *anceps* is topographically one of the most anomalous, in the light of our recent knowledge of the distribution and ecology of this plant. The Duggerna Rock, situated at the southern entrance to Moore Bay, Kilkee, is a very extensive platform of intertidal rock, measuring approximately 550 m from east to west by about 500 m from north to south. Much of the inner part of this remarkable littoral plateau consists of relatively flat areas of broken rock (with deep pools, etc.) on which all of the fucoids of sheltered places occur, together with such species as

Bifurcaria rotunda (Huds.) Papenf. and *Himanthalia elongata* in deep, sheltered, mid-littoral pools. However, out towards the north-west extremity of the platform (about 320 m from the shore high-water line) the rocks become higher again over a quite small area and stick up as a small rock pinnacle which remains just above the surface at high tide, although waves usually wash over it. It was only on the steep seaward facing side of this 'offshore' elevation that *Fucus spiralis* f. *nana* and *F. distichus anceps* were found in typical narrow belts, although some few plants of *anceps* were found also on two narrow, exposed reefs immediately west of the elevation. Sometimes this particular offshore elevation is referred to as *the* Duggerna Rock and, judging by his description, this is undoubtedly the precise site at which W. H. Harvey first found *anceps* (see Carruthers, 1863; and Powell, 1957, for further references).

As already stated, however, the configuration of the shore at this site is somewhat different from that of most *anceps* sites in that, immediately to the west of the offshore elevation, lie two narrow reefs which slope gently *inshore*; these reefs are not very extensive and do not rise so high as the main offshore elevation so that they shelter from direct wave-crash only the lower parts of the Duggerna elevation. It was very interesting to find plants of *anceps* on the higher parts of both of these shallow inshore-facing reefs, in each case at a lower level than the *anceps* plants on Duggerna Rock itself.

Because of the unusual shore configuration, the zonation of dominants at this site was somewhat confused, but the salient features were as follows.

The offshore elevation (*Duggerna Rock*). The highest parts (flat) were covered with patches of *Chthamalus* and *Verrucaria* sp., and with frequent *Littorina saxatilis* (Olivi) and *L. neritoides* (Montagu). The western face of the elevation is steeply sloping or near vertical (for a vertical height of 2·7–3·1 m). The usual dominant species of such exposed Irish coasts occurred on this wall in a very open and mixed community, as follows: *Fucus spiralis* f. *nana* mostly in the uppermost 1·8 m (best developed on ledges) and *F. distichus anceps* in the lowermost 1·5 m, together with small plants of *F. vesiculosus* f. *linearis*, tufts of *Gigartina*, etc. The plants of *anceps* were rather small and had mostly ceased fruiting (17 July). Below the wall, on shallow ledges, *F. vesiculosus* f. *linearis* occurred in tufts, with barnacles, *Mytilus*, etc.

The two inshore-facing reefs beyond. The inner of the two reefs was about 18 m broad and, on the lower stretch of 12 m, was dominated principally by *Mytilus* and *Fucus vesiculosus* f. *linearis*, with frequent *Chthamalus*, *Gigartina*, etc., forming a dense closed community; on the higher part of the reef, however, about 6 m broad, these same species formed a much more open community (probably a direct result of severe wave-action) and here *Fucus distichus anceps* was found, either as isolated plants on small patches of otherwise bare rock, or else mixed with the other species in open communities.

The outer reef was about 30 m broad, and on the seaward side its uplifted western edge dipped quite steeply down into deep water; this western edge was dominated by *Alaria*. On the smooth inshore-facing surface of the reef the (lowermost) inner half was dominated by very crowded *Mytilus*, with scattered tufts of *Fucus vesiculosus* f. *linearis*, *Gigartina*, etc.; but throughout the higher half of the reef scattered plants of *Fucus distichus anceps* were again found, mixed with the above and other species of plants and animals in a rather open community.

Thus, on each of the two reefs, scattered plants of *anceps* occurred on the higher (least sheltered) parts only; it is of interest that the plants themselves were quite large (*ca.* 7–10 cm long) and that most were sterile, although some plants had precocious irregular areas of receptacular tissue some distance behind the apices; the deduced age was 1 year—see p. 682). It is presumed that the relatively large size was a result of the plants growing at a relatively lower level on the shore than usual (less desiccation) and that they were able to do so because of the unusual configuration of the reefs at this otherwise very exposed site; however, since only very few fully fertile (2-year-old?) plants were found on the two outer reefs it may be assumed that conditions there are more hazardous (severe wave-action and intense competition from mid-littoral species) than subsp. *anceps* encounters at its more usual level.

It is of interest that on the outer reef one plant of *linearis* was found with several vegetative plants of *anceps* growing epiphytically on it; the two forms were readily distinguishable in the field by means of the midrib character (see p. 670). This is the only record of any form of *F. distichus* growing epiphytically on another large alga.

The remaining sites at which *anceps* was found in south-west Co. Clare are those numbered 4–8 in Text-fig. 3. Most of the intervening stretches of coast were either precipitous and inaccessible from land, or else were too sheltered for *anceps*.

Site 4 (Text-fig. 3)

This is a broad reef, several 100 m long, on a very exposed coast just to the west of Bridge of Ross (a natural arch of rock); the reef again faces north-west so that oceanic swell approaching mainly from the south-west tends to run along as well as slightly up the reef. At the more sheltered north-east head of the reef, the belts of some of the dominant plants and animals were again obviously uplifted relative to theoretical tide-levels, but there was much evidence that the high-level belts of algae were very adversely affected (stunted and desiccated) by summer air temperatures.

The reef sloped very evenly, at an angle estimated to be *ca.* 10°. The lower shore was dominated by an uplifted belt (*ca.* 7·5 m broad) of sparse *Alaria*, merging upwards into a very open belt (*ca.* 3 m broad) of mixed *Gigartina*, *Mytilus*, *Corallina officinalis*, *Patella aspera*, *Nemalion elminthoides* (Vell.) Batt., *Callithamnion arbuscula*, *Polysiphonia macrocarpa* Harv., small *Scytosiphon lomentaria* and (unusually for an *anceps* site) frequent small plants of *Laurencia pinnatifida* (Huds.) Lamour., with *Chthamalus* largely confined to small pits and cracks in the otherwise very smooth surface of the rock. *Fucus distichus anceps* occurred in the zone next above (up to *ca.* 7·5 m wide), mixing with the above-named species and some few plants of *F. vesiculosus* f. *linearis* below, and with *F. spiralis* f. *nana* above; the maximum width of the *nana* belt was *ca.* 3·5 m. Both fucoid belts were very open. The plants of *anceps* appeared to comprise two distinct year-groups: (i) larger plants, mostly in the centre of the belt, which were in a late fruiting stage (2 years old?) and covered with brown epiphytes; (ii) younger vegetative plants (1 year old?) mainly above and below the belt of older plants, and with few or no epiphytes. The older plants particularly were very dried out on a warm day (18 July) and the epiphytes borne on the arched fronds had stuck to the rock in many instances. Above the two fucoid belts occurred a belt (*ca.* 7·5 m broad) of sparse and stunted *Porphyra* with *Chthamalus* (and some *Pygmaea pumila* (Huds.) Kuntze and *Blidingia minima*), while *Chthamalus* continued farther upshore for approximately 11 m, but strictly confined to crevices and altogether very inconspicuous. On the open rock in the upper part of the *Chthamalus* belt, patches of dried-out *Bangia fuscopurpurea* (Dillw.) Lyngb. and Myxophyceae indicated that earlier in the year algae had flourished at much higher levels on the open reef than they could in summer. *Littorina saxatilis* and *L. neritoides* continued in crevices very much higher upshore than *Chthamalus*. The theoretical range of spring tides in this area is about 4·3 m and from very approximate estimations it seems that most of the *anceps* belt probably occurred below the theoretical level of M.H.W.S. Unfortunately, *Balanus balanoides* was extremely scarce and no estimate could be made of its 'general upper limit' to assist comparison of the levels occupied by *anceps* in Ireland and Scotland. However, there is little doubt that *anceps* generally occupies a somewhat lower zone (relative to both theoretical and effective mean high water levels) in south-west Co. Clare than it does in Scotland.

Toward the more exposed and wave-beaten south-west end of this same reef, the zones of dominant species were uplifted to a greater extent and first *anceps* and then *nana* were lost from the flora.

Fucus distichus anceps occurred also on the next reef immediately to the north.

Site 5 (Text-fig. 3)

Here very good belts of *anceps* and *nana* were found on a rather steeper reef (estimated slope 15–20°), with the same associated dominant species above and below. At this site *anceps* again occurred in two distinct forms, apparent year-groups, the larger having mostly ceased fruiting and occurring mainly in the lower half of the *anceps* belt.

Site 6 (east of site 5)

Here the littoral area is for the most part very steep (30–40°), but with flat ledges of rock at intervals; *anceps* was very well developed, especially on the ledges, and occurred in a zone at least 1·5 m in vertical extent (directly measurable at this site). Plants of the two apparent year-groups (see p. 682) were again present in about equal numbers, and at this site both young and old plants occurred throughout the 1·5 m range. In addition, many crowded groups of *anceps* sporelings occurred on *Chthamalus* and on rock in the *anceps* belt. This reef was examined during a heavy rain-storm, and it was noted that some *anceps* plants were growing in a depression completely submerged by a temporary strong stream of fresh water. Some specimens of *Balanus balanoides* were noted among the frequent *Chthamalus* in the *anceps* zone. It was again observed that *nana* persisted farther than *anceps* along the steeper and more wave-beaten part of this shore.

Site 7 (Text-fig. 3)

This site is extremely difficult of access, with a steep descent to a very exposed littoral zone. The reef is small in area, mostly steep in the lower littoral and affected by violent swell; however, the upper littoral area flattens out to a shallow slope and, at this level, on the more exposed part of the reef, good belts of *Fucus spiralis* f. *nana* and *Gigartina* (below) were developed. *Fucus distichus anceps* occurred below the *nana* only on a slightly more sheltered part of this restricted high-level platform; the plants were small and some were still in a late fertile stage.

Goleen Bay (Text-fig. 3, site 8)

F. distichus anceps was found in quantity on the outer reefs of the rocky promontory at the northern entrance to the bay. The reefs at this site slope very steeply (20–30°) down into deep water, but they mostly face due north so that swell from the south-west again tends to run along them rather than directly upshore. The characteristic zonation of dominant species on the more exposed rock faces was as follows: *Chthamalus* on the upper shore; then *Chthamalus*/*Porphyra umbilicalis*; *Fucus spiralis* f. *nana*; *F. distichus anceps*; *Gigartina*, etc.; *Alaria*. The site was apparently too exposed for *Fucus vesiculosus* f. *linearis*. Again, *nana* was more tolerant than *anceps* of increasing exposure. The *anceps* formed an almost closed community and occurred in three distinct colour shades: (i) light yellow—young, first-year vegetative plants; (ii) russet brown to orange—old plants, just ceasing to fruit; (iii) dark brown—old plants densely covered with epiphytic *Elachista fucicola* (Vell.) Aresch. and *Spongonema tomentosum* (Huds.) Kütz. Both young and old plants occurred throughout the *anceps* belt, and in general the plants of both age-groups were smaller towards the top of the belt. Epiphytes were most dense on the lowermost old plants. A large number of *anceps* sporelings were again observed, on *Chthamalus* and on rock, in the *anceps* belt.

The inner part of Goleen Bay is very sheltered and the shore consists of mixed boulders and reefs, covered with all the usual fucoids of sheltered Irish shores. Since *anceps* occurred in unusual abundance at the entrance to the narrow bay it was considered that, if sporelings of Irish *anceps* were able to develop into larger plants (approaching subsp. *edentatus* in form) under sheltered conditions, the inner part of Goleen Bay would be one of the most likely places to find such plants. No plants of *F. distichus* were found in the inner parts of the bay, however. Further, no intermediate forms between *anceps* and *edentatus* were found anywhere in Ireland.

At Baltard Bay (see Text-fig. 3) the shore configuration is very similar to some of the least steep Caithness reefs, and such reefs are extremely rare in the west of Ireland. The angle of slope was estimated as probably not more than 5° and the reef extends as an almost unbroken sheet of smooth Millstone Grit, from the base of low cliffs well above effective high-water level right down into deep water. The reef faces west and oceanic swell, running in along the southern flank of Baltard Bay, sweeps directly upshore. As in Caithness, these conditions result in a considerable uplift of the belts of dominant species, but the shallow reef is so extensive that the upper shore is not subjected to anything like the severity of swell and wave action experienced on the lower shore. Thus *F. vesiculosus* f. *linearis* was found to be well developed in the upper mid-littoral zone (mostly small, narrow plants), with *F. spiralis* f. *nana* frequent above it; subsp. *anceps*, however, was absent. The principal differences at this site, compared with sites of similar configuration in Caithness, were seen on the upper shore: *Chthamalus* was now the dominant barnacle and extended upshore (usually confined to cracks and hollows in the rock) for a considerable height above theoretical mean high-water level; and the high-level belts of *Porphyra* (above *nana*) and Myxophyceae were not nearly so well developed in summer (although *Porphyra umbilicalis* was abundant in the effective mid-littoral zone on such Irish shores).

FRUITING RECORDS

Among the records and collections of subsp. *anceps* reported above, fertile plants have been found in all of the months from April to August. Only sterile plants were collected at Kilkee in 'September 1897' (by E. George); and J. Sinclair likewise found only sterile plants at Hole o'Rowe, Orkney, on 16 October 1938.

The only plants collected in the month of April (island of Lewis-Harris, *W. J. Gibson*, 1909—see p. 667) were fully mature, so it may be supposed that receptacles start to develop at least as early as January and probably a little earlier.[1] However, even though it is not known when fruiting starts, it is certain that it largely ceases during August at all sites. There is a suggestion that the peak of fruiting is reached some few weeks earlier near the southern limits of distribution (the Kilkee district) than in the Scottish localities.

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[1] Börgesen (1902) reports that in the Faeröes fertile specimens of *Fucus distichus* were found from April to August; specimens gathered in October and November were sterile; in December a few specimens were found bearing young receptacles. However, Börgesen does not state which form of the species was examined in December.

POSSIBLE LONGEVITY

The observations on populations of *anceps* in south-west Co. Clare in July 1953 strongly suggest that there this plant has typically a 2- to 3-year life-span. Thus, at several sites, it was obvious that two distinct populations occurred intermixed: (i) relatively large, fully mature plants, frequently with all the apices fertile (or else with old receptacles dying back), and bearing many mature brown epiphytes (principally *Elachista fucicola* and *Spongonema tomentosum*); and (ii) shorter, vegetative plants, usually quite sterile, or else with only one or two (precocious) receptacles (often just part of a frond some way back from the apex), and with few or no epiphytes. Usually populations (i) and (ii) were quite intermixed, but occasionally, on reefs of even slope, belts of (ii)-type population occurred principally above and below a crowded main belt of (i)-type population. At several sites, crowded groups of really small sporelings were found in the *anceps* belt; length of sporelings (0·5–)4–10 (–13) mm, with one or two dichotomies and with prominent cryptostomata. It is thought that these small sporelings were probably not more than a few months old, population (ii) 1 year old, and population (i) 2 years old. After fruiting, the receptacles of the presumed 2-year-old plants die back and, since often no vegetative leaders are left to continue growth, it seems that the whole of such a plant usually dies back and is removed from the rock by wave-action during the third winter of its life. Indeed, vestiges of holdfast and stipe were seen at several sites in Ireland. However, some of the mature (i)-type Irish plants examined did show a few remaining vegetative leaders that could continue growth into a third year if the plants are able to survive the storms of a third winter; these leaders are usually lateral branches issuing from quite low down on the stipe.

At the Scottish sites, sterile (1-year-old) plants were again distinctive at all sites, but the fertile plants (probably at least 2 years old) commonly bore quite a number of vegetative leaders (again issuing mainly from the proximal rather than the distal parts of the plants) and it is probably more usual for plants to survive for at least 3 years, especially on many of the Caithness reefs where conditions are not quite so rigorous as at some of the other Scottish sites.

DISCUSSION

Fucus distichus subsp. *anceps* thus shows a very discontinuous distribution in Britain and Ireland, being confined to some few isolated and usually very limited stretches of exposed coast in the north and west. The various centres of distribution are mostly geographically isolated from each other by considerable distances, and locally the species is ecologically confined to very exposed reefs, that are often difficult of access.

At all the *anceps* sites described above, all or most of the littoral area of the shore slopes evenly and more or less steeply (5–40°) down into deep water, usually without any major offshore protection, and with the 5 and 10 fathom (9 and 18 m) isobaths usually close inshore. All of the sites are also subjected to constant and frequently violent oceanic swell, as well as severe wave-action, which sweep a long way up shores of this configuration and result in a marked upward extension of the littoral zone as a whole. The conditions of life for species growing in the upper littoral at such sites are obviously more rigorous than on more sheltered shores for, although frequently inundated during rough weather, and wetted by spray from breaking waves and swell even more often, there will be periods of relatively calm weather when the uplifted high-level species must suffer severe desiccation, especially in the warmer summer months, unless frequent rain or mist mitigates these conditions. In general, the desiccation will be a good deal more severe at the warmer southern (Irish) sites than at the cooler northern (Scottish) sites. Fucoids are only able to survive on such wave-beaten shores at the higher levels, where they develop into narrow dwarf forms such as *F. spiralis* f. *nana* and *F. distichus anceps*, and it is interesting that in Ireland the former with the more southerly over-all distribution tolerates rather more rigorous exposure conditions than the latter 'northern' species; whereas at the Scottish localities *anceps* persists farther into exposure than *nana*. It is very difficult to make comparisons of levels occupied by the same dominant species on shores such as these (with differing tidal ranges, aspect, angle of slope, etc.), but there were some indications that the two belts of high-level fucoids occur at relatively rather lower levels at some of the Irish sites than they do at the Scottish sites, and this could be a result of more severe desiccation at the Irish sites. Indeed, it seems that the eventual southern limit of *F. distichus anceps* is probably determined by increasingly severe desiccation at the high level occupied, retreat downshore being limited by the more severe wave-action and intense competition from other plant and animal species in the zone below.

This type of smooth shore configuration is very rare, and most of the exposed rocky shores of Britain and Ireland are very much more broken up. This usually means that much of the force of swell and wave-action is dissipated either on off-shore reefs or islands or, more often, in the lower littoral, with the result that the over-all height of the littoral zone is not raised to anything like the same extent; indeed, the upper shore is often comparatively sheltered from wave-action under these conditions, as is best indicated by the species that usually grow there (e.g. *Pelvetia canaliculata*). On such broken or flatter coasts, with intermediate exposure conditions prevailing at least in the mid- and upper littoral zones, *Fucus vesiculosus* f. *linearis* commonly dominates the mid-littoral in Britain, with a zone of *F. spiralis* (or *F. spiralis* f. *nana*) above it.

Ecologically then *F. distichus anceps* appears to be confined to exposed sites (with extended littoral zone) largely as a result of competition from more successful forms of *F. vesiculosus* and *F. spiralis* on coasts of intermediate exposure; and intermediate forms of *F. distichus* (between subsp. *anceps* and subsp. *edentatus*) are largely absent from British coasts of intermediate exposure (except for a few instances in Caithness) probably for the same reason. It is very interesting that farther north, nearer to one of the centres of distribution of *F. distichus* (e.g. in the Faeröes, Iceland and northern Norway), intermediate forms of the species are commonly found on coasts of intermediate exposure and, although a form of *F. vesiculosus* without vesicles occurs commonly in these places, it is apparently restricted to more sheltered sites than in Britain (see Börgesen, 1902, p. 478; 1905, p. 720 *et seq.*).

Additional records of *F. distichus anceps* may be expected from certain restricted stretches of very exposed coast that have a similar configuration and ecology to that described above for the various sites known to date. However, in view of the over-all distribution, it is considered most unlikely that the form will be found anywhere else on the *mainland* of Britain other than on the north coast of Scotland. Additional records will probably come only from exposed coasts in the following areas: the Shetland and Orkney Islands, the Outer Hebrides (and possibly Tiree and Islay), and the north and west coasts (only) of Ireland. Even within these areas, however, the number of possible sites physically, and hence ecologically, suitable for *F. distichus anceps* are remarkably few as can be seen from large-scale maps and charts. Indeed, several of the Irish sites for *F. distichus anceps* were predicted from a study of fairly large-scale maps (1 in. to 1 mile, with contours, gives sufficient detail).

FUCUS DISTICHUS L. EMEND. POWELL, SUBSP. *EDENTATUS* (DE LA PYL.) POWELL (Pl. II, fig. 2)

DISTRIBUTION

In Britain, *F. distichus* subsp. *edentatus* has been found at the following three localities only: Lerwick and Scalloway, Shetland Islands, and North Haven, Fair Isle (Text-fig. 1).

In reporting the presence of the species at Lerwick in 1902, Börgesen (1903, p. 304) stated that 'it grew here at about low-water mark on the quay itself, as well as on stones opposite to it; but in spite of a very close search I did not succeed in finding it anywhere else, neither to the south-east of Lerwick, nor in the sound near Bressay, nor [at] Burra Voe, nor on Muckle Holm' (Yell Sound).

According to the printed label attached to specimens of *edentatus* distributed by E. M. Holmes as '*Fucus inflatus* Vahl' (*Alg. Brit. Rar. Exsicc.*, Fasc. XII, No. 288), another collection of plants from Lerwick harbour was made in 'June 1908' by 'W. A. Russell'.

In June–July 1952, *edentatus* was found to be still abundant in the harbour at Lerwick and was found also at the following two new localities—in the shelter of the small harbour at Scalloway, a small fishing town not far from Lerwick, and in the sheltered North Haven on Fair Isle, which is the usual harbour for the island. The Fair Isle record represents a new southern limit for this form of the species on the British side of the North Sea and a preliminary notice of the record (under the name *F. inflatus* f. *edentatus*) was given in Burrows *et al.* (1954).

ECOLOGY

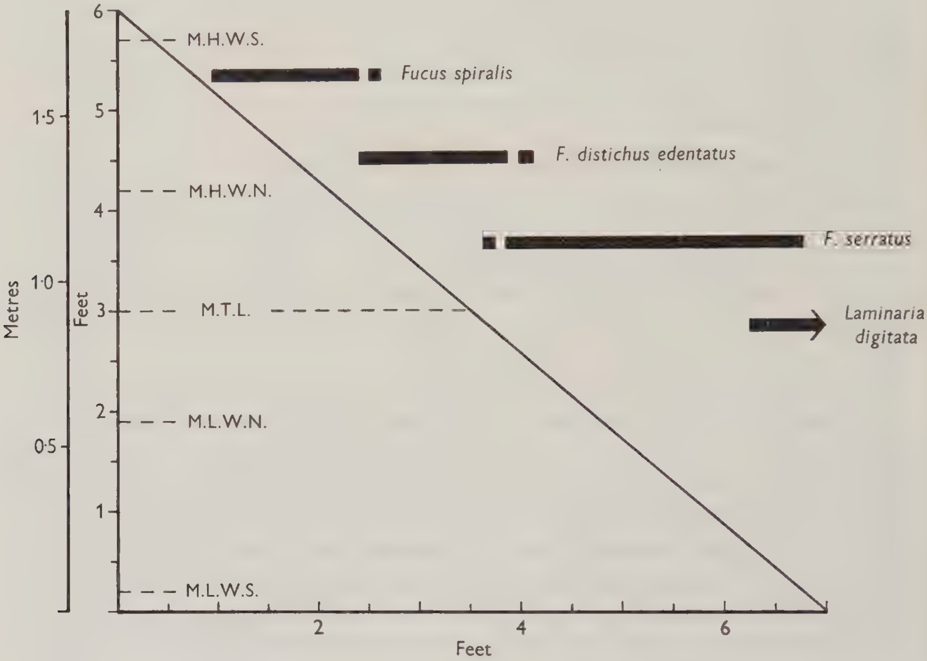
Lerwick, Shetland

Of the three localities mentioned, *F. distichus edentatus* was best developed in Lerwick harbour proper. Here there is a tidal range of only 1·68 m at spring tides and 0·76 m at neaps (*Admiralty Tide Tables*, 1956). The stone-built walls of the inner harbour (along the sea-front) have an average slope of *ca.* 40–45° and in 1952 were largely dominated by a dense growth of fucoids, but along most of the wall only three (fucoid) species were present (Text-fig. 5).

F. spiralis occupied a belt 30–45 cm in vertical extent, in the upper part of the littoral; *F. serratus* dominated a zone, 60–90 cm in vertical extent, on the lower shore (above *Laminaria digitata* (Huds.) Lamour); and between these two fucoids occurred a belt (*ca.* 30–45 cm in vertical extent) of *Fucus distichus edentatus*, slightly overlapping the belts above and below. Small plants of *Pelvetia* were occasionally present above the *Fucus spiralis* belt, but *F. vesiculosus* and *Ascophyllum nodosum*, usually so abundant on sheltered shores in Britain, were quite absent—their usual position being occupied by *Fucus distichus edentatus*. In the British Isles this zonation pattern is unique to Lerwick harbour, where it occurred on vertical as well as sloping walls. Tracing the fucoids south-eastwards beyond the built-up walls of the harbour proper, it was found first that, on a more open (but still comparatively sheltered) and gently sloping shore (of natural reefs, boulders and sand), near Queen's Hotel (Leag beach), *edentatus* occurred down to lower levels and the individual plants were then larger; again *F. spiralis* occurred on the upper shore and *F. serratus* (here with very deeply incised serrations) on the lower shore, and no other fucoids were present. Continuing south-eastwards along the shore, however, *F. vesiculosus* quite soon appeared in the mid-littoral zone and, as it increased in amount, so the *F. distichus edentatus* decreased until, within a few hundred metres of Lerwick harbour, it disappeared from the flora. (*Pelvetia* appeared just before *Fucus vesiculosus*, and some plants of *Ascophyllum* were found with the first *Fucus vesiculosus*).

On the Leag shore especially, plants of *edentatus* frequently had irregular inflations in the distal parts of the fronds, and sometimes grew in shallow pools. *F. serratus* also frequently had inflated apices at this site (observed on a rather warm day).

These observations at Lerwick could suggest that *F. distichus edentatus* may compete with *F. vesiculosus* and *Ascophyllum* for the available space in parts of the *outer* harbour area, but on the *inner* harbour wall the complete replacement of the other two species by *edentatus* is altogether unusual, and is almost certainly not entirely (and perhaps not at all) the result of competition. There is very considerable pollution in Lerwick harbour caused particularly by effluent pouring directly into the harbour from the fish factories that line the quays, and it would seem that *edentatus* is more tolerant of this pollution than is either *Fucus vesiculosus* or *Ascophyllum*. It is interesting that Lund (1949*a*, *b*) also has noted that *Fucus distichus edentatus* in Copenhagen harbour shows greater tolerance of polluted water than does *F. vesiculosus* or *F. serratus*; the individual plants of *edentatus* were also smaller at such polluted localities (Copenhagen) than at less polluted places nearby.



Text-fig. 5. Diagram to show zonation of the dominant algae on the wall of the inner harbour at Lerwick, June 1952, and the approximate relation of the algal belts to the mean tidal levels there (mean levels derived from *The Admiralty Tide Tables*).

Scalloway, Shetland

During a brief visit to Scalloway on 3 July 1952, patches of rather small plants of *edentatus* were found in the sheltered harbour. The plants occurred on a gently sloping shore composed of boulders, shingle and sand, at the western head of the bay between the two piers. The tidal range is small (only 1·13 m at springs), and it is probable that these waters were again somewhat polluted. The fucoids present in open communities on this rather unstable shore were: *F. spiralis* forming a belt on the upper shore; small amounts of both *F. distichus edentatus* and *F. vesiculosus* intermixed in the middle part of the littoral; and plentiful *F. serratus* on the lower shore. The small plants of *edentatus* had almost ceased fruiting, and many plants had irregular inflations. It was most unusual (for Britain) to find that nearly all of the plants of *F. serratus* had inflated apices.

North Haven, Fair Isle

In the sheltered North Haven on Fair Isle (a small and predominantly sandy bay), *edentatus* was again not very abundant compared with Lerwick, although it was found on rocks or boulders at both the east and west sides of the bay, on the boat-slip at the centre of the bay and on the stone landing jetty in the south-east corner of the bay. The tidal range is 1·74 m at springs, and the Haven is not polluted. On the west side of the Haven the rock face above the sandy beach is very steep or vertical; here *F. spiralis* occurred in the uppermost 30–45 cm of the *Balanus balanoides* belt, and occasional plants of *Fucus distichus edentatus* were found below the *F. spiralis* belt and farther down to about M.T.L.; the lower plants of *edentatus* were larger than those growing higher up; no other fucoids occurred on this steep face, the lower levels being occupied by *Gigartina stellata*, *Acrosiphonia centralis* and *Enteromorpha* spp.

The boat-slip in the centre of the bay is made of concrete and iron and runs down the sandy beach into deep water. The cement was well covered with *Balanus balanoides*; *Pelvetia* and *Ascophyllum* were absent; on the inshore end of the sides of the slip occurred a belt of *Fucus spiralis* (30–45 cm vertical extent) and below this a belt of *edentatus* (30–38 cm vertical); farther out on the slip and at a lower level, *edentatus* occurred mixed with *F. vesiculosus* and occasional *F. serratus*, with an undergrowth of *Gigartina*, *Lomentaria articulata* (Huds.) Lyngb., etc.

In the south-east corner of the bay the nearly vertical sides of the stone-built landing jetty showed the following zonation of fucoids: a narrow belt of *Fucus spiralis* above, then a belt of *F. vesiculosus* but with a small amount of intermixed *Ascophyllum*, and finally some plants of *Fucus distichus edentatus* on the lower part of the wall.

On the shore nearby (just a few metres to the west of the jetty) the zonation of the dominant algae was worked out in detail and is recorded in Burrows *et al.* (1954, see especially Figs. 4, 5). The shore here sloped very gently; the upper part consisted of a broken rocky reef and the lower part of rounded boulders and rock outcrops embedded in sand. *F. spiralis* formed a good belt (38–45 cm vertical extent) on the reef; *F. vesiculosus* and some *Ascophyllum* dominated the mid-littoral rock and boulders (0·9–1·2 m vertical extent), with associated *Gigartina*, *Cladophora rupestris* (L.) Kütz, *Enteromorpha* spp., *Acrosiphonia centralis*, etc.; while *Fucus distichus edentatus* (large plants) occurred only on boulders on the lower shore, together with *F. vesiculosus* and some *F. serratus*.

Just beyond the jetty, the eastern shore of the Haven (rock reefs and boulders) is very broken, and here only one small group of plants of *edentatus* was present, on the lower shore.

Thus *edentatus* appeared to be fairly well-established in the North Haven: in the most sheltered corner (near the landing jetty) frequent *F. vesiculosus* and some *Ascophyllum* dominated a zone below *Fucus spiralis*, and *edentatus* was confined to more open communities on boulders embedded in sand on the lower shore; but, at the rather more exposed (also steep) sites in the bay, *edentatus* appeared to be more successful than *F. vesiculosus* in colonizing the zone immediately below *F. spiralis*.

FRUITING RECORDS

The plants collected at Lerwick by F. Börgesen in 1902,[1] and by W. A. Russell in June 1908, were both fertile; otherwise records are limited to the 1952 observations when, during the period 24 June–3 July, fully fertile plants were noted at Lerwick, Scalloway and North Haven; at Scalloway, however, most of the receptacles were old and the fruiting period appeared to be nearing an end.

DISCUSSION

These three records of *F. distichus edentatus* represent the southern limit of this form of the species on the British side of the North Sea; the southern limit on the eastern shores of the North Sea is in Copenhagen harbour (Lund, 1949*a*, *b*). In the Faeröe Islands (about 250 miles to the north-west) and in Norway (a similar distance to the east) the plant is plentiful, and it has very probably been introduced accidentally to the three Shetland harbours by the fishing vessels which ply between them, and the Faeröes, and Norway.

There are several ways in which this could happen: pieces of (fertile) *edentatus* could be scraped from a jetty wall by the hull of a fishing vessel (perhaps itself roughened with attached barnacles) and be carried about to some other harbour where the pieces could readily be scraped off against another quay. The chances of subsequent establishment would be increased by the fact that the species is hermaphrodite. Alternatively, as Hylmö (1933) suggests (with reference to the recent spread of the plant on the west coast of Sweden), it could be taken onto fishing vessels, growing on the shells of *Mytilus edulis* (used for bait), and later be jettisoned with the empty shells perhaps in another harbour. Introduction by fishing vessels seems a more likely possibility than introduction by floating plants, although the terminal parts of fertile plants (with swollen receptacles, and often with irregular inflations in the thallus) probably could float and be carried for considerable distances by surface currents.

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[1] In his paper Börgesen (1903, p. 300) states that he visited Lerwick 14–16 July [1902]; a fully mature specimen of subsp. *edentatus* in Herb. Kew, however, is labelled by Börgesen 'Lerwick, 16. 6. 1902'.

It is fairly safe to assume that *Fucus distichus edentatus* has been present in Lerwick harbour for the past 55 years at least. However, we cannot judge from Börgesen's limited observations whether or not the species has increased in quantity in this time; he may not have seen those parts of the inner harbour wall where the species now forms a definite belt between *F. spiralis* and *F. serratus*.

The size and vigour of the British specimens indicate that such physical conditions of the environment as sea and air temperatures do not directly determine the present southern limits of *edentatus*. Perhaps rather the limits to further spread (very locally) are imposed principally by increasingly severe competition from species that are more successful locally. If this is a correct interpretation of the observations, it would be an excellent illustration of Gause's (1934) contention that two species with similar ecology cannot live together in the same place. On a broader scale in northern Britain, the further spread of *edentatus* southwards (to other sheltered sites, where for one reason or another conditions are not favourable for *F. vesiculosus*) is doubtless limited to a large extent by sea-barriers.

If *edentatus* were to spread further in Britain, and if fishing boats are indeed the principal means of dispersion, it might be supposed that the plant would appear next in harbours (especially those that are more or less polluted) in the south of Shetland, in the Orkney Islands (e.g. Kirkwall and Stromness), or even in harbours in north-east Scotland such as at Thurso and Wick. I have looked for the species but not found it in the following likely places. (1) Grutness Voe (south end of Shetland Mainland), 1952. (2) Kirkwall harbour (Orkney), 1952, a brief examination of the vertical harbour wall showed very few fucoids present at all, and much oily pollution. (3) Stromness harbour (Orkney), 1952; a rather more careful search here showed, very interestingly, that *F. vesiculosus* and *Ascophyllum* were absent from the harbour area; on parts of the harbour wall (tidal range 3·08 m at springs) a wide belt of *Fucus spiralis* above merged into a wide belt of *F. serratus* below, but very often there was a 'gap' between the two belts occupied in part by *Balanus balanoides* but quite devoid of fucoids. Stromness harbour appeared to be polluted, and is a likely place for *Fucus distichus edentatus* to become established if ever it were introduced, there being a suitable vacant 'ecological niche'. (4) The harbour at Scrabster (near Thurso), 1951.

There is no evidence to suggest that *edentatus* is actively extending its range southwards in Britain at the present time.

TAXONOMIC CONSIDERATIONS

The distribution and ecology of forms of *F. distichus* in Britain and Ireland, described above, is a good illustration of the fact that, towards the southern limits of distribution, the species is represented by populations of only one or two of the best adapted forms, confined to restricted habitats and often geographically isolated. It may well be that such ecological restriction and geographical isolation (particularly of subsp. *anceps* in Ireland) could result in the gradual evolution of genetically distinct ecotypes or even species.

It is probably safe to assume that *anceps* has been present in Ireland since 1863 at least, and it is highly possible that it may even be a survivor there from a time when the seas around Britain were much colder, but able to exist now only under the extraordinarily narrow range of habitat conditions described in the present paper. It is a well-known phenomenon that species or subspecies that are represented by a single ecotype confined to a limited area frequently exhibit exceptionally small 'ecological amplitude' (i.e. the range of habitat variation that a plant can tolerate; see Daubenmire, 1947) compared with others which, by means of genetic diversification, are represented in a variety of habitats. The apparent genetic impoverishment of these entities may arise by either of two courses of events: (i) catastrophes destroy most of the population and the remnants are *depleted species* or *relicts*, with only limited genetical possibilities for morphological variation; or (ii) an ecotype of small ecological amplitude may result from a very limited crossing of an effective geographical barrier. The population descending from such a single introduction can have no greater genetic heterogeneity than that possessed by the individuals that immigrated (subsequent mutations excluded). Certainly *anceps* shows very limited ecological amplitude in Ireland, but rather more in Caithness. Wherever it occurs in Britain and Ireland, however, *anceps* seems more likely to be a relict form, rather than an introduction of recent centuries. That it may be actively extending its range southwards at the present time is considered very unlikely.

The *edentatus* in Shetland and Fair Isle harbours, on the other hand, has more probably been introduced to those places in comparatively recent times, but certainly before 1902 in Lerwick harbour.

ACKNOWLEDGEMENTS

This paper brings together information from many sources, including field observations made during several surveys of parts of the coasts of north Scotland and west Ireland, and I wish to record my thanks to all the colleagues who participated in the various expeditions—Dr E. M. Burrows, Dr E. Conway, Dr J. R. Lewis, Dr S. M. Lodge, Dr M. de Valéra (Galway), and my wife, Grace Powell—for help of various kinds and for permission to include here some of the observations made on the surveys; these colleagues, however, are not responsible for the views here expressed.

I wish to thank also: Dr Burrows and Dr Lewis for the photographs shown as Pl. II, fig. 2, and Pl. I, fig. 1, respectively; Dr Mary Parke (Plymouth) and Dr A. P. Orr (Millport) for helpful criticism of the typescript; Dr Burrows and Prof. L. A. Harvey (Exeter) for material of *F. vesiculosus* f. *linearis* from Lundy; and Miss C. I. Dickinson (Kew) for help in tracing herbarium specimens.

Finally the author gratefully acknowledges grants received from the Browne Fund of the Royal Society toward expenses incurred in the surveys of the north coast of Scotland and parts of the west coast of Ireland.

SUMMARY

The distribution and ecology of subspp. *anceps* and *edentatus* of *F. distichus* L. emend. Powell (1957) in Britain and Ireland is described in detail, based on recent ecological surveys and critical examination of all past records.

F. distichus anceps is a small form confined to certain very exposed coasts in the north and west of Scotland and Ireland, where it reaches the southern limit of its geographical range. This plant may be a relict form in Britain, existing now only under an extraordinarily narrow range of rigorous habitat conditions; it is not thought to be actively extending its range southwards at the present time. The ecology of subsp. *anceps* is considered principally in relation to that of other reduced fucoids found on exposed British shores, viz. *F. spiralis* f. *nana* and *F. vesiculosus* f. *linearis*; all three forms are illustrated by photographs and means of distinguishing them are described. The life-span of subsp. *anceps* is thought to be 2 to 3 years at some Irish sites, but may be longer at some of the Scottish sites.

F. distichus edentatus is a large, sheltered-water form, and has been found only in the harbours of Lerwick and Scalloway (Shetland Islands) and North Haven (Fair Isle), which mark the southern limits of this subspecies on the British side of the North Sea. It is best developed in Lerwick harbour where it forms a belt between *F. spiralis* and *F. serratus* and appears to be very tolerant of water polluted by effluent from fish factories. *F. distichus edentatus* has possibly been introduced to these three harbours within recent centuries,

perhaps by fishing vessels; it is not thought to be actively extending its range southwards in Britain.

The facts presented show that, at the southern limits of its distribution in Britain, *F. distichus* is represented by populations of only one or two of the best adapted forms, confined to restricted habitats and often geographically isolated. It may be that such restriction and isolation (particularly of subsp. *anceps* in Ireland) could result in the gradual evolution of genetically distinct ecotypes or even species.

REFERENCES

ADMIRALTY, HYDROGRAPHIC DEPARTMENT, 1956. *The Admiralty Tide Tables, Vol. I, European Waters, 1957*, London.

BATTERS, E. A. L., 1902. A catalogue of the British marine algae. *J. Bot., Lond.*, Vol. 40, Supplement, 107 pp.

BÖRGESEN, F., 1902. Marine algae. *Botany of the Faeröes*, Part II, pp. 339–532, pls. XIII–XXIV. Copenhagen (1903). (Issued separately.)

—— 1903. The marine algae of the Shetlands. *J. Bot., Lond.*, Vol. 41, pp. 300–6.

—— 1905. The algae-vegetation of the Faeröese coasts, with remarks on the phytogeography. *Botany of the Faeröes*, Part III, pp. 683–834. Copenhagen (1908). (Issued separately.)

BURROWS, E. M., CONWAY, E., LODGE, S. M. & POWELL, H. T., 1954. The raising of intertidal algal zones on Fair Isle. *J. Ecol.*, Vol. 42, pp. 283–8.

CARRUTHERS, W., 1863. *Fucus distichus* Linn. as an Irish plant. *J. Bot., Lond.*, Vol. 1, pp. 353–5, pl. 12.

COTTON, A. D., 1912. Clare Island Survey: Marine Algae. *Proc. R. Irish Acad.*, Vol. 31, Part 15, 178 pp., 11 pls.

DAUBENMIRE, R. F., 1947. *Plants and Environment. A Textbook of Plant Autecology.* xiii, 424 pp. New York and London.

GAULD, D. T., BAGENAL, T. B. & CONNELL, J. H., 1953. The marine fauna and flora of St Kilda, 1952. *Scot. Nat.*, Vol. 65, pp. 29–49.

GAUSE, G. F., 1934. *The Struggle for Existence.* Baltimore.

GILLHAM, M. E., 1954. The marine algae of Skokholm and Grassholm Islands, Pembrokeshire. *Northw. Nat.*, Vol. 25, pp. 204–25, pls. 3–5.

HARVEY, L. A., 1950. The granite shores of Lundy. *Ann. Rep. Lundy Field Soc.*, Year 4 (1950), pp. 34–44, 1 pl.

HUDSON, W., 1762. *Flora Anglica.* xv, 506 pp. London.

HYLMÖ, D. E., 1933. Algenimmigration nach der schwedischen Westküste. *Bot. Notiser*, 1933, pp. 377–90.

LEWIS, J. R., 1954. The ecology of exposed rocky shores of Caithness. *Trans. roy. Soc. Edinb.*, Vol. 62, pp. 695–723, 4 pls.

—— 1955. The mode of occurrence of the universal intertidal zones in Great Britain. *J. Ecol.*, Vol. 43, pp. 270–90.

LIGHTFOOT, J., 1777. *Flora Scotica.* 2 Vols. London.

LUND, S., 1949*a*. Nye Alger for de danske Farvande. (With summary in English.) *Bot. Tidsskr.*, Bd. 48, Hft. 3, pp. 231–53.

—— 1949*b*. Immigration of algae into Danish waters. *Nature, Lond.*, Vol. 164, p. 616.

NEWTON, L., 1931. *A Handbook of the British Seaweeds.* xiii, 478 pp. London.

POWELL, H. T., 1957. Studies in the genus *Fucus* L. I. *Fucus distichus* L. emend. Powell. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 407–32.

POWELL, H. T. & LEWIS, J. R., 1952. Occurrence of *Fucus inflatus* L. forma *distichus* (L.) Börgesen on the north coast of Scotland. *Nature, Lond.*, Vol. 169, p. 508.

TRAILL, G. W., 1885. *A Monograph of the Algae of the Firth of Forth.* 17 pp. and 4 pp. of mounted algae. Edinburgh.

EXPLANATION OF PLATES

PLATE I

Fucus distichus L. emend. Powell, subsp. *anceps* (Harv. et Ward ex Carruthers) Powell

Fig. 1. Photograph of part of a belt of plants growing on an exposed reef near Lower Dounreay, Caithness, Scotland (site 4 in Text-fig. 2), July 1951, showing characteristic arching of the fronds. (Photograph J. R. Lewis.)

Fig. 2. Photograph of a typical mature Caithness plant (preserved in formalin/seawater, but not pressed), collected just west of Sandside Head, Caithness (site 2 in Text-fig. 2), July 1951, *H. T. Powell* (MILL). Greatest length of plant 17·4 cm; most of the apices are fertile; plant probably *ca.* 2 years old. The scale is 15 cm long.

PLATE II

Fig. 1. *Fucus distichus* L. emend. Powell, subsp. *anceps*. Photograph of a living mature plant from a reef just east of Brims Ness, Caithness (site 9 in Text-fig. 2), June 1956, *Mrs Grace Powell* (MILL). Greatest length of plant 23·7 cm; receptacles up to 5·7 cm long and up to 6 mm broad; some of the branches have been cut off. This is the longest plant of subsp. *anceps* found so far in Britain and it was growing on a reef slightly sheltered from direct wave-crash; all of the apices were fertile. In certain respects the plant is intermediate in form between subsp. *anceps* and subsp. *edentatus* (see p. 672).

Fig. 2. *F. distichus* L. emend. Powell, subsp. *edentatus* (De la Pyl.) Powell. Photograph of a mature plant growing on a fairly sheltered shore at Leag, just to the south of Lerwick harbour, Shetland Islands, 3 July 1952. Some plants of *F. spiralis* are present in top right background; the barnacles are *Balanus balanoides*. (Photograph E. M. Burrows.)

PLATE III

Fig. 1. *Fucus distichus* subsp. *anceps*. Same plant as in Pl. I, fig. 2, with some of the branches cut off, spread out and photographed under conditions similar to those for the lower illustration. Note that in the distal branches the midribs are scarcely distinct from the alae.

Fig. 2. *F. vesiculosus* L., f. *linearis* (Huds.) Powell. Photograph of a very reduced plant (preserved in formalin/seawater, but not pressed), from very exposed coast at Jenny's Cove, Lundy, Bristol Channel, *L. A. Harvey* (MILL). April 1949, with young receptacles. Greatest length of plant 14·4 cm. Some of the branches have been cut off. Note the very distinct midribs in the distal branches, and the absence of vesicles (see p. 670).

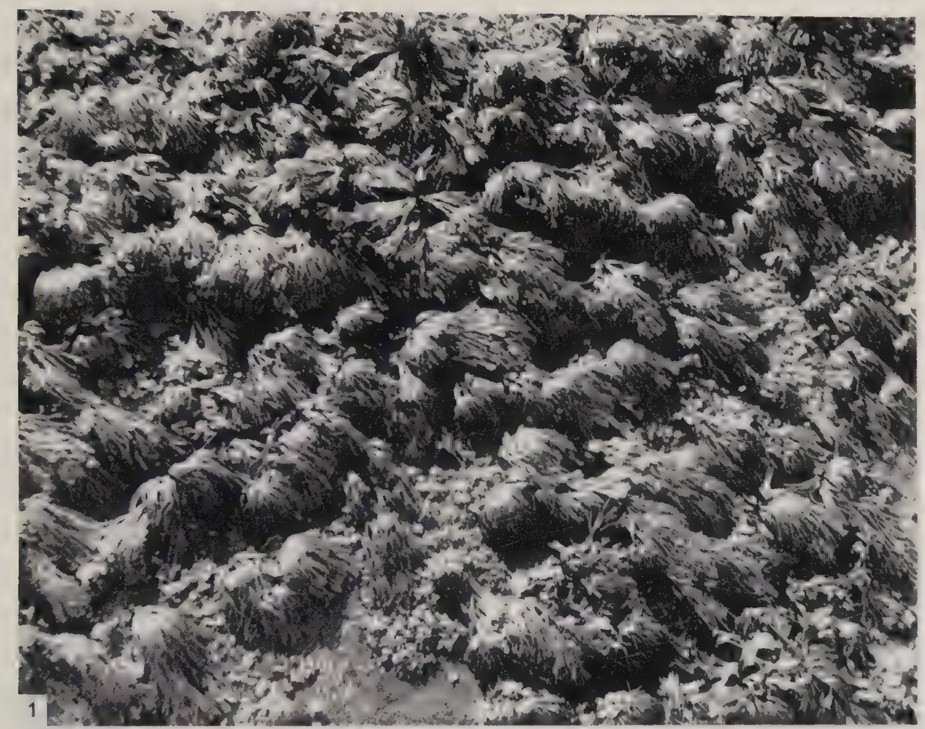
The scales show cm and mm.

PLATE IV

Fig. 1. *Fucus vesiculosus* f. *linearis*. Photograph of a very reduced plant (preserved in formalin/seawater, but not pressed) from exposed coast at Jenny's Cove, Lundy, Bristol Channel, August 1950, *L. A. Harvey* (MILL). Greatest length of plant 15·0 cm. Note the long, narrow receptacles, most of which are mature, the prominent midribs and the absence of vesicles (see p. 670). Some of the branches have been cut off.

Fig. 2. *F. spiralis* L., f. *nana* (Stackh.) Batt. Photograph of several plants (preserved but not pressed) from exposed reefs near Lower Dounreay, Caithness (site 4 in Text-fig. 2), July 1951, *H. T. Powell* (MILL) (see p. 671). Greatest length of right-hand plant 12·9 cm.; most of its apices are fertile.

The scales show cm and mm.



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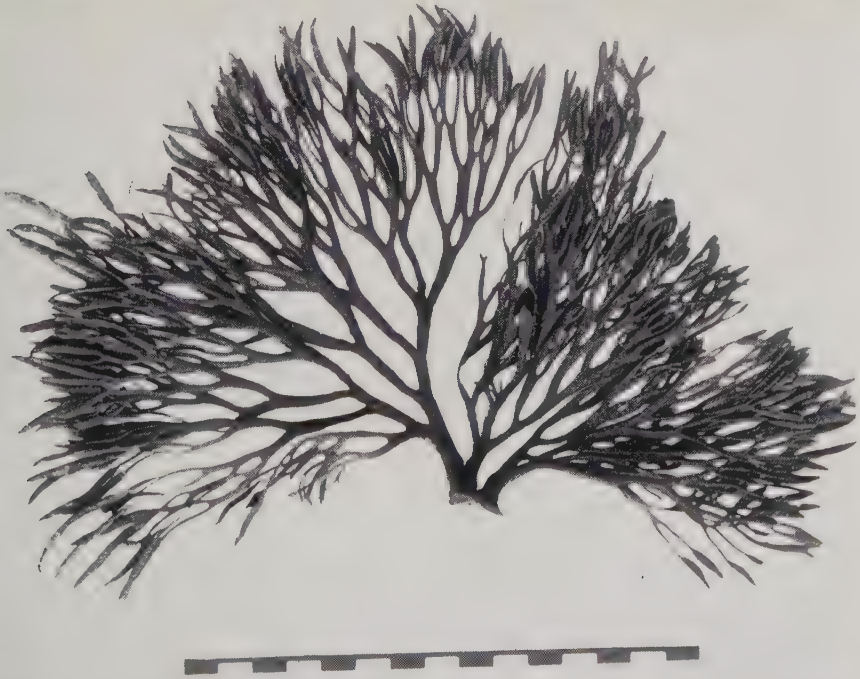

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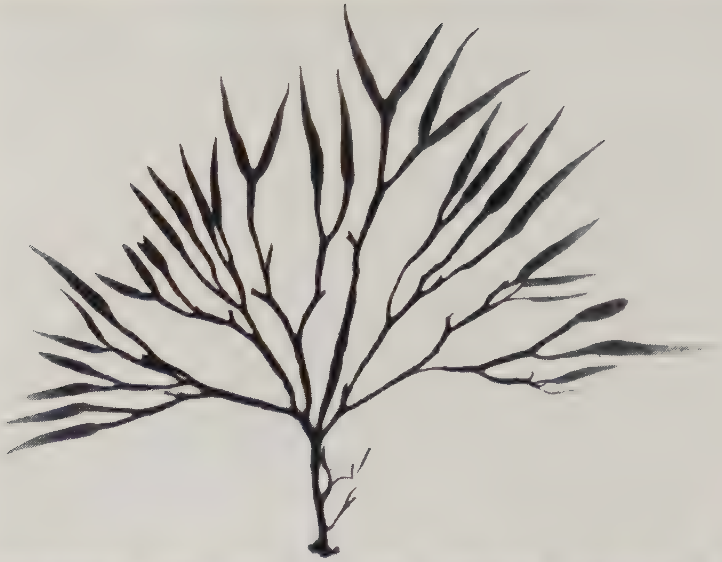
(*Facing p.* 694)



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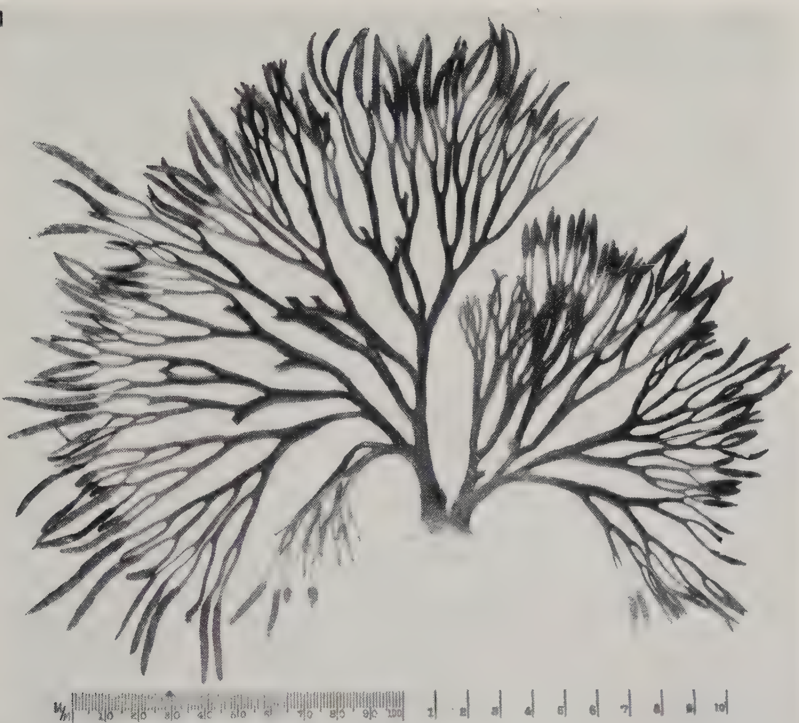

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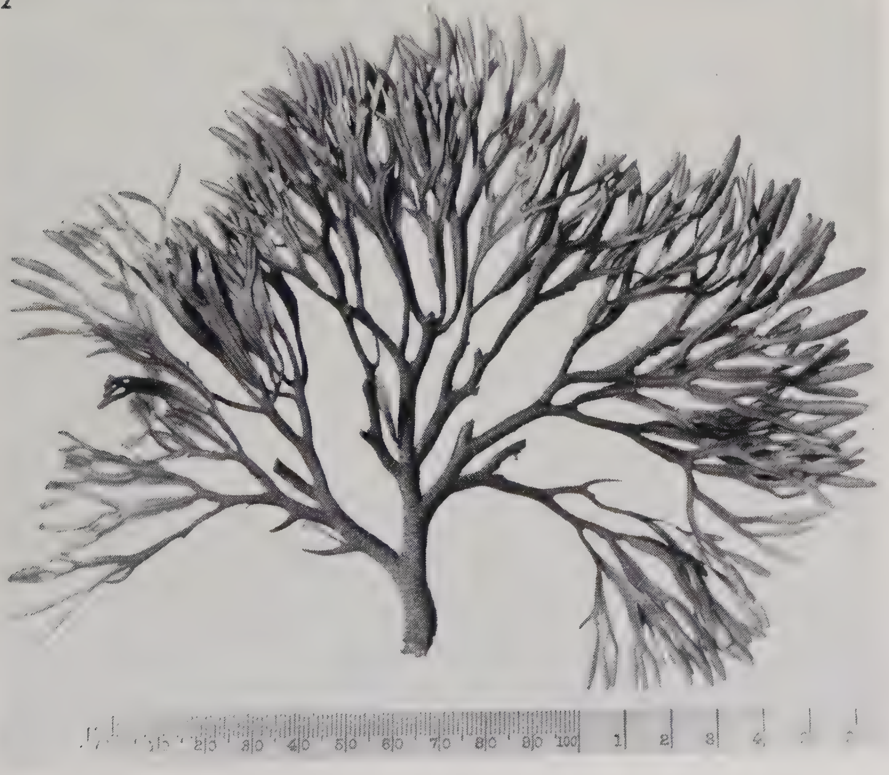

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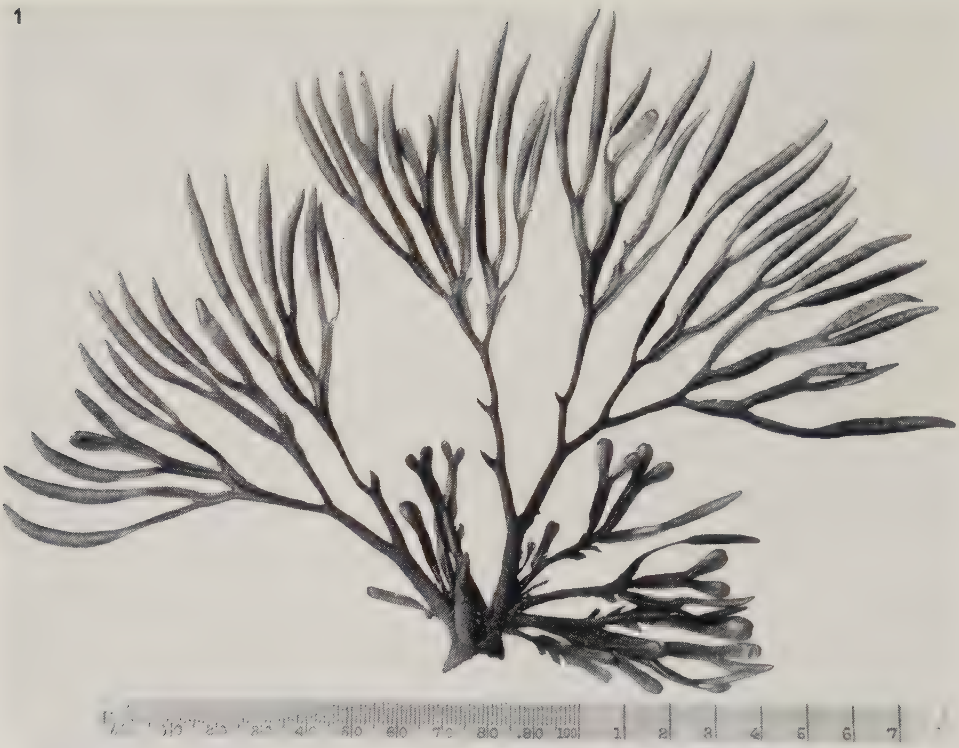
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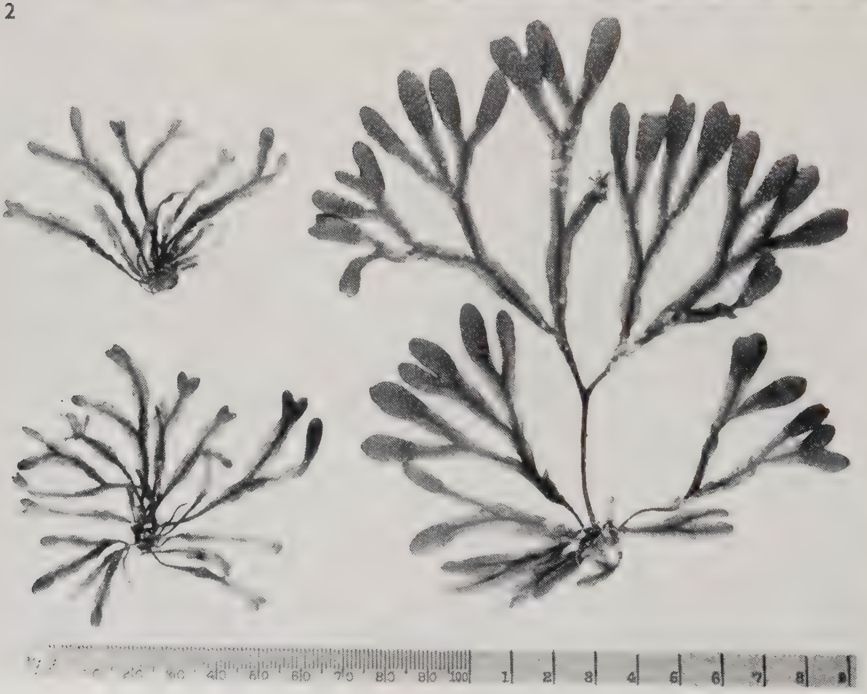
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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

RED BLOOD-CELL ANTIGENS IN SOME LOWER VERTEBRATES

By DOREEN E. ASHHURST

J. exp. Biol. 1956, Vol. 33, pp. 249–55

The main aim of the work described in this paper was to discover if blood groups could be found in Amphibia and fish using human blood grouping techniques. No evidence for blood groups could be found in the whiting, pouting, plaice or frog by cross-matching the red cells and serum of animals of the same species. Some frogs were injected with the red cells of other frogs but no antibodies to red cells could be detected in the injected frogs. The experiments suggest that, in these species, all the animals used had the same antigens on their red cells.

The red cells of frogs, toads, tree-frogs and newts were tested against human ABO sera. All the sera (anti-G(A), anti-G(B), anti-G(A+B) and AB serum) reacted with the red cells of each species, but with the Anuran red cells, the reactions with anti-G(B) and anti-G(A+B) were much stronger. The weak reaction is caused by species antibodies present in all human sera, but the strong reaction was shown to be due also to the presence of a B-antigen on the Anuran red-cells. In frogs the B-antigen is identical with the human B-antigen. D.E.A.

TOXIC MARINE FLAGELLATES; THEIR OCCURRENCE AND PHYSIOLOGICAL EFFECTS ON ANIMALS

By DOROTHY BALLANTINE and B. C. ABBOTT

J. gen. Microbiol. 1957, Vol. 16, pp. 274–81

The distribution of toxic water blooms in the sea and brackish water is discussed and illustrated by a map. This distribution picture is not complete but gives indications of the cosmopolitan nature of the phenomenon. The causative organisms have also been listed as far as possible, and a brief discussion of the causes and economic importance of red tides follows.

The latter half of the paper is devoted to a discussion of the physiological effects of these toxins, with particular emphasis on the toxin from *Gymnodinium veneficum*. The effect of this toxin is compared with the action of the toxin causing paralytic shellfish poisoning.

This paper is one of a series in a symposium on plankton, held by the Society for General Microbiology, in September 1956, at Exeter. D.B.

THE DIRECT ACTION OF ANTERIOR PITUITARY EXTRACTS ON THE INITIATION OF LACTATION IN THE RABBIT

By D. B. Carlisle

Physiol. Comparata et Oecologia, 1957, Vol. 4, pp. 295–312

Experiments are described which demonstrate that a crude pituitary extract, which has a systemic effect in initiating lactation in the primed male rabbit, does not possess any direct, local action. From such an extract it is possible to prepare a purified hormone which is directly, locally lactogenically active. Some of the implications are discussed.

D.B.C.

LARVAE OF THE BRITISH SPECIES OF *DIOGENES*, *PAGURUS*, *ANAPAGURUS* AND *LITHODES* (CRUSTACEA, DECAPODA)

By J. D. Macdonald, R. B. Pike and D. I. Williamson

Proc. zool. Soc. Lond., 1957, Vol. 128, pp. 209–57

Larvae, obtained both from laboratory hatchings and from plankton, are described for the species *Diogenes pugilator* (Roux), *Pagurus bernhardus* (L.), *P. pubescens* Krøyer, *P. prideauxi* Leach, *P. cuanensis* Thompson, *Anapagurus laevis* (Thompson), *A. hyndmanni* (Thompson), *A. chiroacanthus* (Lilljeborg) and *Lithodes maia* (L.), and a single larva obtained from plankton from off Plymouth attributed to *Pagurus sculptimanus* Lucas. Keys to these larvae are given.

A consideration of all the known larvae of hermit crabs and stone crabs shows that they fall into two main divisions, corresponding to the Coenobitidae plus Diogeninae on the one hand and the Lithodidae plus Pagurinae on the other: a division which can also be made from the disposition of the third maxillipeds of the adults. It is suggested that the classification generally accepted fails to reflect the phylogeny of the various groups, and a revised classification is proposed in which each of the two divisions indicated by the larvae forms a superfamily of the Anomura.

It is also shown that the larvae of the species of *Pagurus* fall into two distinct groups; when further species have been investigated the splitting of the genus might be justified.

D.I.W.

BOOK REVIEW

ALLGEMEINE MEERESKUNDE: EINE EINFÜHRUNG IN DIE OZEANOGRAPHIE

By G. Dietrich

With a chapter by K. Kalle

Published by Gebrüder Borntraeger, Berlin, 1957; Price: DM. 56. Pp. viii+492 (including 223 text-figures), 30 text-plates and 7 loose folding plates.

It is fifteen years since Sverdrup, Johnson and Fleming's '*The Oceans*' appeared. The time is now ripe for another standard text-book of general oceanography. Dietrich has met the need with a book which combines the German gift for accurate detail with clear and concise presentation of broad issues. He has used a direct prose style free from the grammatical tangles which may make the German language so difficult for those who have not mastered it.

His colleague, Kurt Kalle, has contributed a chapter of sixty pages on the geochemistry, biochemistry and productivity of sea water. This is the only chapter into which biological matters enter. The remaining 400 pages are devoted to physical oceanography, all branches of which are covered more than adequately. Where all is so good it is not easy to select one section for special mention. Mathematics is used when necessary but no biologist need fear that he will be overwhelmed by formulation of which he has neither need nor interest. An English translation of this excellent book with such a distinctive approach ought to sell well.

L. H. N. COOPER

MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

Report of the Council for 1956–57

The Council have to report with regret the death of Mr B. Storrow, an Associate Member of the Association since 1930.

THE COUNCIL AND OFFICERS

During the year Major E. G. Christie-Miller resigned from the office of Honorary Treasurer to the Association, which he has held since 1941. The Council wish to record their thanks to Major Christie-Miller and their appreciation of his devoted services to the Association for so long a period.

Mr Harrison S. Edwards has been elected Honorary Treasurer, and it is a source of much gratification to the Council that the office continues to be held by a Governor representing the Fishmongers' Company.

Four ordinary meetings of the Council were held during the year, three in the rooms of the Royal Society and one at Plymouth. At these the average attendance was seventeen. The Association is indebted to the Council of the Royal Society for the use of its rooms.

THE PLYMOUTH LABORATORY

The excavation of the new sea-water reservoirs which began in October 1955 has proved a harder task than originally anticipated. The drilling of the rock was not completed until the end of October 1956.

The final construction of the reservoirs was, however, completed by 31 March 1957, and the building of new outside circulation tanks is well in hand.

The walls of one of the old sea-water reservoirs, which had been leaking, have been relined with a waterproof coating.

AQUARIUM

The aquarium has been open throughout the year and has been well attended. No major structural alterations have been made.

RESEARCH SHIPS

The three research vessels, *Sarsia*, *Sula* and *Gammarus*, have been in regular operation apart from routine surveys.

PLYMOUTH MARINE FAUNA

During the year the new edition of the *Plymouth Marine Fauna* has been in the hands of the printers and it is anticipated that it will soon be ready for publication.

The Council wish to record their thanks to members of the Plymouth laboratory staff and others who have contributed to this new fauna list. The editing of the manuscripts and correction of the proofs have been an exacting and time-consuming work, and the Council wish specially to thank Dr D. P. Wilson on whom this burden has fallen and whose skill and knowledge have been given so willingly, and Mr G. M. Spooner for the help given from his wide faunistic experience.

STAFF

Dr J. S. Alexandrowicz retired from the staff of the Plymouth laboratory on 31 March 1957. The Council wish to place on record their appreciation of the distinguished researches made by Dr Alexandrowicz, and the valuable help he has given to the Association.

Dr E. J. Denton joined the staff of the Plymouth laboratory as Principal Scientific Officer on 1 April 1956.

Dr A. J. Southward joined the staff of the Plymouth laboratory as Scientific Officer on 1 October 1956 in a special supernumerary post.

Mr E. I. Butler joined the staff of the Plymouth laboratory on 1 October 1956 in the grade of Experimental Officer.

Mr F. A. J. Armstrong has been promoted to the grade of Senior Scientific Officer as from 2 April 1956.

Dr D. P. Wilson visited a number of marine biological and other laboratories in the United States in April–May 1956 after attending the Symposium on Marine Biology at the Scripps Institute of Oceanography, La Jolla.

Dr B. C. Abbott attended a colloquium on Marine Biology held under the auspices of the International Union for Biological Sciences at the marine biological laboratory at Roscoff in June 1956.

Dr D. B. Carlisle spent three weeks in April 1956 at Lund University and the Kristineberg Marine Zoological Station.

Dr B. C. Abbott, Dr E. J. Denton and Dr T. I. Shaw attended the International Congress of Physiology in Brussels in August 1956.

Mr G. R. Forster attended the meeting of the International Council for the Exploration of the Sea in Copenhagen in October 1956.

Dr B. C. Abbott has been elected a Fellow of the Institute of Physics.

Mr W. H. Searle was awarded the British Empire Medal in the New Year's Honours for 1957.

Occupation of Tables

The following one hundred and thirty workers have occupied tables at the Plymouth laboratory during the year:

E. Adams, Plymouth (Library).
Dr Daphne Atkins, Plymouth (Ciliary mechanisms of brachiopods).
T. B. Bagenal, Millport (Spawning of plaice).
Miss J. A. Bailey, London (General).
Mlle R. Barreau, Bordeaux (Algae).
Prof. E. J. W. Barrington, Nottingham (Endocrinology of tunicates).
Miss G. Beeson, Oxford (General).
P. E. Benstead, Southampton (Chemistry of sea water).
E. J. Binyon, London (Permeability of tube feet in *Asterias*).
G. T. Boalch, London (*Ectocarpus*).
Q. Bone, Oxford (Primitive chordate nervous systems).
A. D. Boney, Plymouth (Red algae).
Miss P. E. Brockman, Plymouth (Library).
K. H. Bultitude, Birmingham (Sensory physiology of elasmobranchs).
Dr P. C. Caldwell, Beit Memorial Fellow (Biochemistry of muscle and nerve).
Mrs P. C. Caldwell, Plymouth (*Spartina*).
J. W. Coles, London (Nematodes associated with seaweeds).
K. Copeland, London (Electronic equipment).
Mrs R. M. Cornell, Liskeard (Library).
Dr E. D. S. Corner, International Paints Research Fellow (Effects of toxic substances on marine organisms).
C. A. Cosway, Torquay (Library).
Miss W. A. M. Courtney, London (Ecology of cirratulids).
Dr D. J. Crisp, Anglesey (Feeding of cirripedes).
Dr Phyllis G. Croft, London (Humane killing of lobsters).
Prof. Sears Crowell, Indiana (Morphogenesis and growth of hydroids).
Dr R. Phillips Dales, London (Feeding rates in sabellids and serpulids).
E. W. Dawson, Cambridge (Physiology of lamellibranch foot).
R. D. Deshpande, Reading (Physiology of Trochidae).
Miss E. J. Dimelow, Reading (Functional morphology of *Antedon*).
J. T. Dunbar, Kingsbridge (Library).
D. Etherington, London (Library).
Mrs D. Etherington, London (*Ectocarpus*).
Surg.-Lt. R. J. Fallon, R.N., Plymouth (Library).
Dr Maria Felinska, Oundle (Ciliates).
Dr L. R. Fisher, Reading (Vitamin A in zooplankton).
E. Ford, Millport (Library).
Cdr R. H. C. F. Frampton, R.N. (Rtd.), Plymouth (Library).
R. F. H. Freeman, London (Activity cycles in *Scrobicularia*).
Dr Vera Fretter, Reading (Prosobranchs).
Dr D. N. Ganguly, Calcutta (General).
Dr J. B. Gilpin-Brown, Bristol (Nervous system of *Nereis*).
Dr Sylvia J. Gilpin-Brown, Bristol (Behaviour of littoral gastropods)
Dr M. Ginsburg, Bristol (Teleostean neurohypophysis).
Dr D. R. Glasson, Plymouth (Library).
Prof. A. Graham, Reading (Prosobranchs).
P. Gray, Plymouth (Library).

E. R. Hansen, Plymouth (Library).
Dr E. Jean Hanson, London (Histology of molluscan muscles).
Prof. A. C. Hardy, F.R.S., Oxford (Drawings of marine animals).
M. G. Hardy, Reading (Nervous system of ctenophores).
Dr A. E. Harper, Cambridge (Glucose-6-phosphatase in *Lophius*).
F. A. B. Hawes, Poole (Settlement of *Tubularia*).
Dr R. H. Hedley, London (Foraminifera).
B. T. Hepper, Conway (Lobsters).
Prof. A. V. Hill, F.R.S., London (Heat production in nerve).
J. V. Howarth, London (Heat production in nerve).
Dr G. M. Hughes, Cambridge (Respiratory movements of *Scyllium*).
O. D. Hunt, Newton Ferrers (Library).
W. G. Inglis, London (Osmo-regulation and locomotion in nematodes).
Dr C. H. Jellard, Plymouth (Library).
Mrs J. Jellard, Plymouth (Library).
Miss P. M. Jenkin, Bristol (Library).
Dr Margaret W. Jepps, Cambridge (Foraminifera).
W. E. Jones, Anglesey (Algae).
Miss E. A. Kay, Hawaii (Cypraeidae).
Dr G. Y. Kennedy, Sheffield (Marine pyrrol pigments).
Dr R. D. Keynes, Cambridge (Nerve physiology in *Loligo*).
M. C. Kingwell, South Brent (Library).
Dr J. A. Kitching, Bristol (Library).
Sir Francis Knowles, Bt., Marlborough (Colour change in crustaceans).
Miss A. Kuku, London (Axial organs and tube feet in *Echinus*).
Miss B. I. Lade, London (General).
Dr Marie V. Lebour, Plymouth (Decapod crustaceans).
K. Lederis, Bristol (Teleostean neurohypophysis).
Dr J. Llewellyn, Birmingham (Trematode ectoparasites of fishes).
A. R. Longhurst, Freetown (Bottom sampling methods and library).
Prof. O. E. Lowenstein, F.R.S., Birmingham (Sensory physiology of elasmobranchs).
Dr J. Lowy, Belfast (Physiology and histology of muscle).
Dr G. A. C. Lynch, Plymouth (Library).
Miss C. I. MacFarlane, Canada (Brown algae).
Dr Anna Malquori, Naples (*Sepia*).
Prof. Irene Manton, Leeds (Marine flagellates).
E. R. March, Plymouth (Library).
A. L. Martin, London (Feeding and digestion in amphipods).
R. B. Mayne, Plymouth (Library).
P. B. Meurling, Lund (Development of blood vessels in hypophysis region of *Scyllium*).
Prof. N. Millott, London (Light reaction in echinoderms).
Miss F. M. Molloy, London (Digestion in mysids).
Prof. W. F. H. M. Mommaerts, Los Angeles (Heat production in nerve).
Dr J. E. Morton, London (Ecology and physiology of mollusca).
Dr R. W. Murray, Birmingham (Sensory physiology of *Raia*).
Prof. Lily Newton, Aberystwyth (Algae).
Dr E. R. Noble, Santa Barbara (Protozoan parasites of fishes).
Dr C. E. O'hEocha, Galway (Library).
Dr C. F. A. Pantin, F.R.S., Cambridge (Neuromuscular physiology of *Metridium*).
A. H. Papworth, Ashford, Mddx. (Plankton).
Prof. B. Peyer, Zurich (Dentition of fishes).
Miss J. M. Plumtree, Nottingham (Endocrinology of tunicates).

Dr W. T. W. Potts, Birmingham (Ion exchange in lamellibranchs).
P. Reeve, London (General).
Dr A. Revheim, Bergen (Mackerel).
Miss B. Rickard, Plymouth (Library).
Dr F. H. Rigler, Toronto (Ion exchange in *Bryopsis*).
Prof. P. Roa Morales, Venezuela (Chemistry of sea water).
Dr Elaine A. Robson, Cambridge (Neuromuscular physiology of *Metridium*).
Y. S. R. K. Sarma, London (Green algae).
Miss R. S. M. Savage, London (Axial organs and tube feet in *Echinus*).
G. Shelton, Cambridge (Respiratory movements of *Scyllium*).
J. P. Sinha, London (Green algae).
D. S. Smith, Cambridge (*Clunio marinus*).
Miss M. M. Soares, Southampton (Cirripede larvae).
B. W. Sparrow, Newton Ferrers (Library).
Dr A. J. Southward, D.S.I.R. (Distribution and breeding of barnacles).
Dr Eve C. Southward, Plymouth (Polychaetes).
Miss F. A. Stanbury, Plymouth (*Cladophora*).
U. Stefansson, Iceland (Oceanography).
Prof. W. Stephenson, Brisbane (General).
D. H. Steven, Cambridge (Library).
O. Suddaby, Plymouth (Library).
R. V. Tait, London (Plankton).
Dr Marta Vannucci, Sao Paulo (Plankton).
H. Wallace, Oxford (Ascidians).
Miss M. A. Walker, London (Visual pigments of fishes).
P. R. Walne, Conway (Library).
G. E. Walster, Plymouth (Library).
Dr T. Weis-Fogh, Cambridge (Methylene blue staining of nerves).
R. L. Welcomme, London (Library).
Dr Mary Whitear, London (Histology of fish skin and sensory organs in crustaceans).
R. J. Wood, London (General).
M. Yoshida, London (Light reactions in echinoderms and coelenterates).

Among the many scientists who have visited Plymouth during the year to see the general work of the laboratory and to discuss problems with members of the scientific staff, the following have come from overseas: Prof. B. Steinbach (U.S.A.), Dr Gertrud Pleskot (Austria), D. W. LeMare (Penang), Prof. M. Stangenberg (Poland), H. W. Kakyomya (Uganda), Prof. R. P. Forster (U.S.A.), G. Küchler (Holland), Dr R. Buchsbaum (U.S.A.), R. W. Foster (U.S.A.), Prof. W. R. Boss (U.S.A.), Dr G. Filteau (Canada), Prof. C. L. Prosser (U.S.A.), Prof. A. H. Whiteley (U.S.A.), Prof. C. G. Miller (U.S.A.), Miss I. Bennett (Australia), D. L. Worf (U.S.A.), G. H. P. de Bruin (Ceylon), Mr and Mrs S. A. Wainwright (U.S.A.), Prof. K. Suriwata (Japan), J. Kikkawa (Japan), Dr P. E. Thompson (U.S.A.), Dr B. Halstead (U.S.A.), M. R. Ranade (India).

The Easter Vacation Courses were conducted by Mr G. M. Spooner and Mr P. G. Corbin, and were attended by forty students from the following Universities: Oxford, Cambridge, Glasgow, Aberdeen, Edinburgh, London, Durham, Sheffield, Southampton, Exeter, Chelsea Polytechnic and Regent Street Polytechnic.

Also during the Easter Vacation Mr R. J. Jones and Dr C. T. Prime brought a party of ten boys from Whitgift School and Mr J. R. Packham brought seven boys from Varndean School, Brighton.

SCIENTIFIC WORK OF THE PLYMOUTH LABORATORY STAFF

Sea Water and Plankton

Dr L. H. N. Cooper has completed the first stage of his study of the causes underlying long-term fluctuations in productivity of the English Channel. A group of hypotheses has been erected which imply that the deep Atlantic provides the connecting link between fluctuations in Arctic climate and productivity of the shelf waters of Western Europe.

During 1956 precise observations have been made in deep waters off La Chapelle Bank, which is within the range of Decca navigation. Accurate knowledge of depth was ensured by using four unprotected reversing thermometers on a hoist, and oxygen analyses were made in triplicate after randomizing.

The working up of the analyses is not yet completed, but the following deductions may be drawn. (*a*) The muds of the continental slope and abyssal ocean bed are sources of biological oxygen demand; this results in a curtain of partly deoxygenated water blanketing the slope and having no relation with density. (*b*) This curtain seems to be moving with the continental slope on its right, and has vertical and horizontal oscillatory displacements caused by internal waves; these displacements may be large—for example observations three days apart showed changes in oxygen content of up to 1·0 ml./l. (*c*) Strips of this deoxygenated curtain of water may be torn off and form isolated elongated cores surrounded by the well-oxygenated oceanic water. (*d*) The deoxygenated water may have acquired biologically active substances resulting from organisms living in the slope mud and their metabolic products, which may affect the nature and composition of the plankton. (*e*) These investigations and deductions support the direct observations made in a bathyscaphe by Prof. F. Bernard of the University of Algiers who observed strata of almost azoic water lying between layers in which many animals were seen.

This work done in 1956 has strengthened the ideas resulting from the work in earlier years and pointed to new lines. The value to fishery research of a knowledge of the transport of energy across the depths of the ocean and its effects on the distribution of water masses on the continental slope and its adjacent areas does not need stressing.

A paper by Dr Cooper on the use of carbon-14 for assessing the age of oceanic waters has been published in Vol. 35, No. 2, of the *Journal.*

Mr F. A. J. Armstrong has continued monthly cruises to station E 1 and the analyses for phosphate, total phosphorus and silicate. The results for 1955 are being prepared for publication. During the summer of 1956 there have been some rather rapid fluctuations in the silicon content of offshore water. At the same time the salinity of the water has been higher than 35·4‰, which is unusual.

Analyses for iron by the new digestion method have been done regularly at E 1 and five 'L' stations. The results, which now cover nearly two years, show a marked seasonal variation, more iron being found in the winter months. This is thought to be due to greater turbulence and land drainage at this time of year. A few more analyses of deep water have been done, and a method of storing samples without change for a short time has been found.

Some trials of an indophenol method have shown that it can be used for routine determinations of ammonia in sea water after low-pressure distillation. The method will be used for analysis of water from E 1, where it seems that phosphate has not in recent years been the limiting nutrient for phytoplankton growth.

Further analyses of sea water for aluminium by the improved method have shown that the quantities present are too small for sufficiently precise measurement, and a more sensitive method must be found.

The prototype absorptiometer designed by Mr Armstrong has had further tests at sea, and he is now constructing an instrument for regular use, either in the laboratory or aboard ship. A method of checking zeros is incorporated, which simplifies correction for drift caused by battery voltage changes.

Dr D. B. Carlisle and Dr B. C. Abbott have continued their investigations on the uptake of niobium and vanadium by certain ascidians, and the presence of these substances in the sea. Mr L. G. Hummerstone has improved the colorimetric techniques for niobium analysis and has estimated the niobium content of sea water. In collaboration with Dr T. I. Shaw, these investigations have been extended to include a study of the adsorption of radio-active niobium and caesium upon various surfaces under varying conditions of ionic strength. The experiments have shown that whereas sodium and niobium compete for adsorption sites, sodium cannot discharge caesium from most surfaces.

Dr H. W. Harvey has followed the rate at which the cellular carbon increases in cultures of *Phaeodactylum tricornutum* during exponential growth in bright and in dim light, and also the effect of periods of darkness on the subsequent rate of growth. The change in cellular carbon was assessed from the decrease in total carbon dioxide in the medium, which was calculated from the increase in hydrogen-ion concentration.

The experiments are indicating that when growing with ample nutrients and in sufficient light to allow maximum rate of growth, the exponential rate depends upon the chlorophyll content of the cells. That is, the rate of chlorophyll production limits the rate of carbon assimilation when 'light saturated'. In turn the rate of chlorophyll production appears to be the resultant of the rate of (light independent) synthesis minus its rate of photochemical breakdown.

When a 'light saturated' culture growing at a regular, and reproducible, rate was kept dark for twelve hours its chlorophyll and other pigments increased; then when transferred to the same light intensity its cellular carbon increased at an enhanced rate for twelve hours or more, thereafter falling to the regular rate for that temperature and light intensity. Likewise, if a culture growing exponentially in dim light, the cells then being richer in chlorophyll than when growing in bright light, was transferred to 'light saturation', then the increase in cellular carbon proceeded more rapidly for a time than the regular rate for that light intensity and temperature.

The second paper by Dr Mary Parke, in collaboration with Prof. Irene Manton and Mr B. Clarke, of Leeds University, on new members of the Chrysophyceae has been published in Vol. 35, No. 2, of the *Journal*. The study of a further group of these new forms, maintained in culture at Plymouth, should be completed soon. In addition to showing a different type of surface scale covering, these new forms also differ from any previously described in the behaviour of the haptonema (the retractile filiform appendage used for the temporary anchorage of the cell); attachment can occur right along its whole length—not just at the swollen distal end—suggesting that the haptonema in these forms may be ribbon-shaped. Prof. Manton is continuing to collaborate with Dr Parke in the electron microscopy.

The collection of unialgal cultures of marine phytoplankton organisms has had to be maintained throughout the year by Dr Parke and Miss Ballantine owing to the illness of Miss I. Adams. Large numbers of cultures, at least two a week, have been distributed for research purposes to institutions in this country and abroad, in addition to the large volumes of cultures that have been prepared for use by scientists working in the laboratory.

'Corrections and Additions, 1953–1955' to 'A Preliminary Check-List of British Marine Algae' (1953, Vol. 32 of the *Journal*) has been compiled by Dr Parke and published in *Phycological Bulletin*, No. 4, pp. 23–31, May 1956.

Miss D. Ballantine's descriptions of the two new species of *Gymnodinium* (*G. veneficum*, which is toxic to fish and other animals, and *G. vitiligo*) have now been published in Vol. 35, No. 3, of the *Journal*, and she is continuing her work on dinoflagellates.

In February 1956 she visited the East African Colonial Fisheries Laboratory in Zanzibar and was there for one month, during which time she collected numerous tow-net samples of phytoplankton and is still examining these for dinoflagellates. Some work was also attempted on living plankton there, but as there was no means of temperature control, this was almost impossible.

The work of Dr B. C. Abbott and Miss Ballantine on the toxin from *Gymnodinium veneficum* was published in Vol. 36, No. 1, of the *Journal*. This covers the action of the dinoflagellate culture on various animals; the extraction and partial concentration of the poison and study of its action on isolated preparations of hearts, nerves, muscles and frog skin. It has been demonstrated that the poison acts on the nervous system, and depolarizes excitable tissues, probably by interfering with the sodium exchange mechanism. Comparable experiments have been made using a Canadian extract (from shellfish which have become poisonous after eating certain flagellates), which is responsible for paralytic shellfish poisoning in man. This poison is different from that of *Gymnodinium veneficum*. It is hoped to carry out further experiments using the shellfish toxin to ascertain its mode of action and also to carry further the purification of the *Gymnodinium* toxin.

The study of flagellate toxins has practical value since they cause mass mortality of fish in the sea and under cultivated conditions.

Dr E. D. S. Corner, International Paints Research Fellow, in collaboration with Mr B. W. Sparrow (International Paints Research Laboratory, Newton Ferrers), has completed a preliminary investigation of the modes of action of copper and mercury as poisons to certain planktonic crustaceans, and the results of this study have been published in Vol. 35, No. 3, of the *Journal*.

In the course of more recent work determinations have been made of the toxicities of an homologous series of alkylmercuric halides to larvae of *Artemia salina*, nauplii of the barnacle *Elminius modestus* and adults of the copepod *Acartia clausi*. The ethyl ether: sea water partition coefficients of the same series have also been measured. It has been found that although toxicities to both *Artemia* and *Elminius* increase logarithmically as the series is ascended, much larger differences between toxicities are observed when *Artemia* is used than when the compounds are tested with *Elminius*. In addition, a close correlation has been found between the toxicities of organomercurials to *Artemia* and their partition coefficients, a finding consistent with the view that an important factor influencing the toxicities of these compounds to this animal is lipoid solubility. Further experiments are at present being carried out in order to examine the importance of chemical interaction with proteins as a further factor influencing the toxicities of organomercurials, and preliminary studies have been concerned with the relative abilities of these compounds to inhibit urease.

A continuation of this research may lead to an understanding of some of the factors governing the limitation of different species of plankton animals to certain types of water, e.g. oceanic and coastal.

Mr F. S. Russell has continued his observations on deep-sea medusae collected on the cruises of R.V. *Sarsia*. Descriptions of the two species of Scyphomedusae *Nausithoë atlantica* and *N. globifera* have been published in Vol. 35, No. 2, of the *Journal*. Two new species of anthomedusae, *Amphinema* *krampi* and *Merga reesi*, and one new leptomedusa, *Tiaropsidium atlanticum*, have also been described in Vol. 35, Nos. 2 and 3, of the *Journal*.

The 1956 catches of the 2 m stramin ring-trawl showed no departure from the prevailing low level of macroplankton production and *Sagitta setosa* continued as the dominant chaetognath: Mr P. G. Corbin examined the catches.

In co-authorship with his wife, Dr D. P. Wilson has published in Vol. 35, No. 2, of the *Journal* an account of the strandings of *Ianthina janthina* (L.) which took place mainly along the north coasts of Cornwall and Devon in August 1954 during a sustained period of strong westerly winds. Letters to *The Times* and other newspapers obtained the co-operation of members of the public in forwarding reports and specimens from a wide area. These have enabled the extent of the strandings to be plotted and the writing of an account which is unique in the literature of this relatively little-known mollusc, of which no stranding of comparable magnitude appears to have taken place on English coasts for fifty years. Measurements of all the undamaged shells obtained showed that while there is considerable variation in the width of shells of the same height, the width becomes proportionately less with age. From this it is practically certain that small wide shells previously described as separate species (e.g. *I. planispirata* Adams & Reeve) are young specimens of *I. janthina* (L.). These observations confirm and extend the conclusions, based on other evidence, of the Danish author Laursen. Some living snails in excellent condition were also obtained and these enabled observations to be made on float building, reproduction, and some other aspects of the activities of the living animal.

Macro-Flora and Fauna

The reorganization of the herbarium of marine algae has now been completed by Dr Parke and Miss Ballantine and a number of additions have been made to it during the year.

Following his work with the larvae Dr Wilson has made some preliminary observations on the burrowing of adult *Ophelia bicornis* into sands of different types. These experiments can only be regarded as exploratory, intended primarily to lead to a satisfactory technique, and have not reached a stage where it is possible to discuss results.

In readiness for his attendance at the Symposium on 'Perspectives in Marine Biology' arranged by the Scripps Institution of Oceanography and held at La Jolla, California, in March 1956, Dr Wilson spent some time during the preceding months preparing his contribution. This took the form of a paper, entitled 'Some Problems in Larval Ecology Related to the Localized Distribution of Bottom Animals', in which were reviewed several aspects of larval development and settlement about which more knowledge is desirable, as for instance the necessity for paying more attention to the micro-biology of bottom soils in relation to the distribution of the larger species. The paper was well received, and it is understood that it will eventually be published along with other contributions to the Symposium.

In collaboration with Dr J. E. Morton of Queen Mary College, London, and Mr A. D. Boney of the Plymouth Technical College, Dr Corner has made a study of the respiration, rate of filtering and resistance to desiccation of the intertidal bivalve mollusc, *Lasaea rubra*, an animal which may be particularly useful in providing a regular supply of material for future toxicity studies. It is hoped that details of recent work concerning aspects of the ecology of this animal will shortly be published. Preliminary studies of the respiration of the hydroid *Tubularia* have shown that this important fouling organism may also serve as suitable material for toxicity experiments.

Mr G. M. Spooner has continued his work on three of the temperate-zone species of clunionine marine gnats. The early stages of *Thalassomyia frauenfeldi*, not hitherto studied in this country, are being compared with the two existing published accounts on material from the Adriatic and Black Sea respectively. There are differences in both the larval mouthparts and the adult male genitalia which contrast the British population with that of the other two areas, and suggest a subspecific distinction. The larvae live in *Enteromorpha* from H.W.S. to below mid-tide; they are now known to be abundant on the concrete blocks protecting the outer face of the Plymouth Breakwater and to occur even on the Eddystone Rock.

The growth of the larvae of marine chironomids occurs in a few instars with a large jump in size at each moult. Measurements of the head capsule of a collection of larvae of *Cricotopus* (one of the two orthocladine genera with marine species) showed that the instars are absolutely separate in size, the ratio between the dimensions of successive stages being about 1·6.

Study of the occurrence of the commensal polynoid *Harmothoë lunulata* has continued. Specimens have now been obtained in *Phascolosoma elongatum* burrows, and these appear to be indistinguishable in pattern from those of larger size found with *Amphitrite johnstoni*. There is evidence for the existence of at least three independent *lunulata* populations on the Devon and Cornwall coast which seem to have the status of 'host races', familiar among some insects.

Further local collections have been examined of amphipods and isopods for faunistic information. Attention has been given to the amphipod *Isaea elmhirsti*, a little-known epizoic species that lives on the lobster (*Homarus gammarus*), lurking at the bases of the thoracic limbs. Hitherto unrecorded from southern Britain, it proves far from common, but up to five have been found on one lobster. The undescribed adult male is evidently less numerous than the female, but two examples have now been obtained for description.

Study has begun of the Amphipoda from deep water stations in collections made by R.V. *Sarsia*. The species and their relative numbers are being systematically recorded, with an eye for possible links between the distribution of certain forms and that of water masses.

Mr N. A. Holme has continued his work on bottom-living animals, and in July 1956 made a successful bottom-sampling cruise off the south-west coast of Ireland. The continental shelf is here fairly narrow and it was possible to work a line of stations from the shallow water of Dingle Bay out almost to the 100-fathom line. These quantitative samples are of considerable interest since the sea conditions off this coast would only very rarely enable such work to be done.

Mr G. R. Forster has continued work on the fauna of sublittoral rocks. A further twenty-five dives have been made during the summer. Some variations in the sessile fauna in relation to depth and degree of exposure to wave action have been found. For instance, various species of erect bryozoans usually replace ascidians and sponges as the most abundant group when a particular level of shelter is reached. But one of the commonest species, the little madreporarian anemone *Corynactis viridis*, with attractive pink, green, orange and brown colour patterns, is found both in sheltered and exposed conditions and also over the whole depth range so far studied.

New records of sponges include: *Stelletta lactea*, *Stryphnus ponderosus*, *Microciona strepsitoxa* and *Antho involvens*; these together with notes on other rare species have been included in the new fauna list.

The spotted goby referred to in last year's report has been found to be fairly generally distributed in rocky areas near Plymouth and not restricted to the one place from which specimens were taken last year. A waterproofed robot camera, with a flash bulb holder, capable of taking under-water pictures at any distance between 8 inches and 5 feet, has been tested.

Dr A. J. Southward has continued his studies on the distribution, breeding and physiology of barnacles and other intertidal animals. The routine observations at Plymouth and other places in south-west England have shown no marked changes in the barnacle populations during the year, whereas the northern species *Balanus balanoides* had increased in abundance in previous years. The paper on the distribution of intertidal animals and plants along the Channel, prepared in collaboration with Dr D. J. Crisp of Bangor, has been completed after a visit to the Channel Islands.

Dr Southward has finished the study of the breeding of the lugworms *Arenicola ecaudata* and *A. branchialis*, undertaken in collaboration with Dr Eve C. Southward. The former species breeds nearly all the year, ceasing only during the two coldest months, whereas the southern species *A. branchialis* ripens during the summer and spawns mainly from October to December.

During a cruise of R.V. *Sarsia* to the continental slope in the vicinity of 48° 30′ N., 10° W., Dr Southward and Mr Forster dredged up many specimens of the deep-sea barnacle *Hexelasma hirsutum* Hoek from 600 to 700 fathoms. The behaviour of this barnacle in the laboratory suggests that its occurrence may indicate the presence of deep-sea water currents, and further work is being carried out in collaboration with Dr Cooper.

Observations on the Lucernariidae have been continued by Mr P. G. Corbin. Perfect conditions resulted in an exceptionally low spring tide on one occasion in August, making it possible to collect over a large area of the shallow channel dividing Looe Island from the mainland shore: this part is not usually accessible even on a good low spring tide. In the channel, *Halyclistus* was common as on the *Zostera* beds higher up the shore, but in addition *Lucernaria campanulata* was numerous, whereas usually only two or three specimens occur on the *Zostera*. This finding was useful confirmation of the hitherto rather slowly accumulating evidence that this species is distributed from low springs downwards into the sublittoral zone and is not so successful intertidally as *Haliclystus*.

Since H. W. Chang recorded *Callionymus reticulatus* in British waters, there has been some difficulty in separating the 'off-season' males and the females of this species from small *C. lyra* of the same size: the third species, *C. maculatus*, is readily distinguished. Mr Corbin has observed that living specimens of *lyra* and *reticulatus* can be recognized by characteristic differences of their tail markings, of the patterns of spots along the side of the body and of the melanophores on the under-side: these differences hold good for specimens down to 3·0 cm long.

Dr G. A. Steven's work has been badly curtailed and interrupted by serious illness. Nevertheless, he has been able to work up data compiled on otter trawl fish surveys by Dr R. S. Clark and Mr E. Ford in 1919 and the early 1920's (1920, 1921, 1922) and comparative data collected by himself, Mr A. D. Mattacola, and Captain C. A. Hoodless in the early 1950's (1950, 1951, 1952). The area concerned is roughly bounded by a line drawn in a wide sweep from Stoke Point to the Eddystone Lighthouse and thence to Polperro. It appears that on the whole the fish fauna has remained remarkably constant, the 1920's being, if anything, slightly more productive than the 1950's. A few species, previously plentiful, have now, however, all but disappeared from the area. These are *Capros aper*, *Molva molva*, *Squalus acanthias* and *Trigla gurnardus*. Five species have markedly increased in numbers—*Cepola rubescens*, *Merluccius merluccius*, *Mullus surmuletus*, *Pagellus centrodontus* and *Trachurus trachurus*.

Apart from these early data by Clark and Ford, no accurate records of demersal fish fauna in the vicinity of Plymouth have subsequently been kept. Since 1953, therefore, Dr Steven, assisted by Mr A. D. Mattacola and Capt. W. J. Creese, has been recording the catches taken as nearly as possible at weekly intervals in an hour's haul at international station L 4, which is located about mid-way between Rame Head and Eddystone Lighthouse. Already these data are providing interesting and useful information on the rise and decline of year-classes in certain fishes and on the variations in times of appearance and disappearance of migratory species.

During his mackerel investigations Dr Steven has found that best fishing by commercial fishing vessels never occurs in the area of maximal spawning activity. Early in April 1955 (in association with Mr L. B. Pradhan of India) a five-day visit was made to the spawning grounds in the Celtic Sea during which an echo-sounding survey of the area was made in addition to plankton sampling for the collection of eggs and larval stages. Mackerel may evade capture during maximal spawning activity, either because they do not 'swim' when spawning or because they are too deep to be taken in drift nets. Conclusive evidence in support of either hypothesis was not obtained; and a second visit to the area in April 1956 could not be undertaken because of illness. It is hoped to continue the investigation at some future date.

Physiology of Marine Organisms

Much information is available on the life and habits of organisms in the sea and on the chemical and physical factors of their environment. But in order to understand how the organisms react to their environment it is necessary to know much more of their physiology. This is the largest lacuna in the science of marine biology and a wide range of physiological problems are now being investigated at Plymouth.

It is well known that certain brown sea weeds concentrate iodine in their tissues. Dr T. I. Shaw is investigating the mechanism involved and the function of the iodine in *Laminaria digitata*, using the radioactive isotope. The liberation of elementary iodine from iodide outside the weed has been demonstrated, and evidence has been adduced to show that it is this elementary iodine, or a related oxidation state, which is involved in the uptake of the radioactive element. Compounds inhibiting iodine uptake in the weed are known to block the iodine uptake in thyroid tissue.

Further study has indicated that the accumulation of iodine in the weed is closely associated with the oxidative metabolism of carbohydrates, and it has been found that approximately one to two iodine atoms are taken up for each hexose molecule broken down. It has also been found that the respiration rate during the uptake of iodine is very rapid, approaching that found in mammalian tissues at 38° C.

Dr D. B. Carlisle has continued work on the endocrinology of crustaceans, particularly on colour change and on sexual endocrinology. On the former aspect he has continued in collaboration with Mme M. Dupont-Raabe of the Sorbonne, Paris, and with Sir Francis Knowles of Marlborough. A preliminary account of some of the results of this collaboration has appeared in *C. R. Acad. Sci., Paris*, where it is shown that one at least of the colour change hormones of *Leander* contains peptide linkages essential to its function. A visit to Sweden to work in collaboration with Dr R. Fänge and Dr E. Östlund led to further information being obtained on the chemistry of colour change hormones. A recent advance in this field has been the isolation and characterization of yet another chromactivating hormone precursor from the

X organ-sinus gland complex. Like the previously isolated precursors it is not destroyed by boiling and is more stable than most hormones.

Work on the physiology of growth and breeding of prawns is proceeding. The ovarian inhibiting hormone is now the subject of chemical investigation by Prof. Sir Alexander Todd of Cambridge and by Dr R. K. Callow of the National Institute for Medical Research, Mill Hill, both of whom are collaborating with Dr Carlisle. Preliminary results suggest that the hormone may be a steroid. Injection of various known steroids into prawns has been without effect on the ovaries, but this series of experiments is far from complete, since many more steroids remain to be tried. Dr Carlisle has found no evidence for the existence of a testis inhibiting hormone in prawns, despite an intensive search. The only operation which has led to inhibition of testicular function has been to block the vasa deferentia with plasticine plugs. While he was in Sweden, Dr Carlisle took the opportunity of investigating the endocrine basis of sex reversal in the red prawn *Pandalus borealis*. This he found to be essentially similar to that in *Lysmata seticaudata*, but he was able to carry his observations further. It seems evident that sex reversal is controlled primarily by the action of the X organ-sinus gland complex in secreting the ovarian inhibiting hormone, which appears to be the only hormone concerned. This hormone, or rather the cessation of its secretion, is responsible for the control of the onset of sex reversal. The actual assumption of the female form seems to take place at the moult following the attainment of a minimum ovarian size. The degeneration of the testis which follows sex reversal is probably to be attributed to the blocking of the opening of the vasa deferentia which takes place when the animal develops a female shell with the genital openings on the third rather than the fifth thoracic segment.

The moulting process in *Ligia oceanica* is apparently under the same type of control as in the Natantia, according to evidence presented by Dr Carlisle in Vol. 35, No. 3, of the *Journal*. The interval between the two halves of the moult, however, appeared to be outside this control, and no trace was found of any mechanism which might regulate it. He has investigated further the differences in moulting and its regulation in populations of prawns from different localities, and has been examining in the field the moulting and biology of *Maia squinado*. In this species moulting takes place only during a short period of the year, when moulting animals congregate in heaps, apparently for protection. Underwater observations of such a heap have proved fruitful of information.

Dr Carlisle has identified one of the heart-beat regulating hormones (reported by Alexandrowicz & Carlisle in 1953 in the *Journal*) as an *ortho*-dihydroxy indole alkylamine, probably 5,6-dihydroxy tryptamine. A preliminary account has appeared in the *Biochemical Journal*.

Dr J. S. Alexandrowicz has continued his study on the innervation of the heart of the prawn *Leander serratus*. The general arrangement of nerve elements follows a pattern similar to that of other crustaceans. The local system is made up of nine ganglion cells which are all alike. This fact strengthens the contention that in other decapods these cells, although occurring in two sizes, are basically of the same sort. It has also been found that in *Leander* nine arteries, two more than in other decapods, arise from the heart. Each of these vessels has valves supplied by a special set of nerve fibres.

The observations on the histological structure of muscle tissue in crustaceans has led to the following conclusions: (*a*) that the muscles are not composed of units like the muscle fibres of vertebrates, but consist of bundles of myofibrils anastomosing with each other; (*b*) that in the thorax and the abdomen two sorts of muscle tissue with fine and coarse cross-striation are present: the former makes up the bulk of the muscle system which consists of bundles which are usually twisted, whereas the latter forms certain weak muscle units with bundles lying parallel and never twisted.

Dr Alexandrowicz has also studied the innervation of the heart of some molluscs. In the lamellibranchs, *Pinna fragilis* and *Pecten maximus*, it has been found that the two nerves reaching the heart at its anterior part run through the ventricle anastomosing with each other and giving off branches supplying the whole muscular wall; they do not seem to pass to the auricles which are supplied by separate nerve fibres. No ganglion cells have been found in the heart wall.

In *Sepia officinalis* the heart nerve arising from the commissure of the visceral nerves has been found to penetrate the heart wall and appear on its inner surface as a wide trunk the branches of which ramify abundantly and spread over the whole muscular network of the heart. As in lamellibranchs, neither in the ventricle nor in the auricles could ganglion cells be found. From the course of the nerve fibres it would appear that the cell bodies of a good part of the neurons supplying the heart are situated in the branchial ganglion from which some of the cell axons pass directly to the auricles, whereas those of the ventricle run up to the visceral commissure to curve here and enter into the heart nerve. Other fibres of the latter come straight from the central nervous system.

In collaboration with Dr Mary Whitear of University College London Dr Alexandrowicz has investigated nervous organs in the legs of decapod crustaceans. Groups of cells of several types, some containing more than 100 cells in a row, have been observed at the leg joints. Their special features and arrangement in various segments have been determined in several species. A muscle receptor of hitherto unknown type presumably controlling the position of the coxopodite in relation to the thorax has been found in the five appendages in *Homarus*, *Palinurus* and *Eupagurus*, but only in the chela of the crabs, *Maia*, *Cancer*, *Carcinus* and *Portunus*. It consists of a thin muscle attached proximally to a rod-like processus of the endophragmal skeleton and inserting distally into the coxopodite. It has a complicated innervation composed of fibres of various sorts, most of them ending on a dense entanglement.

It has also been found that on the chitinous apodemes projecting into some of the leg muscles nerve cells are present. It is conjectured that they may be elements of the sensory apparatus of the moulting process.

Apart from his work with Miss Ballantine on flagellate toxins, Dr B. C. Abbott has continued his research on the comparative physiology of invertebrate muscle, especially on the lamellibranch molluscs. This work has been done in collaboration with Dr J. Lowy of Queen's University, Belfast. The results of research on the adductor muscle of *Pinna* have recently been published in Vol. 35, No. 2, of the *Journal*, where the mechanical properties of the muscle at rest and during activity are reported. Two papers have been written on the mechanical properties of smooth muscles, one dealing with the general properties of resting lamellibranch muscle and the other with tension changes following stretch of resting muscle (stress relaxation); one of these papers has been published in the *Proceedings of the Royal Society*. The results of experiments on active smooth lamellibranch muscle are being worked up: it is probable that the long sustained closure of the two valves in the mussel and other bivalves is due to tetanic activation and very slow relaxation, rather than to a catch mechanism.

Research has also been carried out on the opacity changes in squid muscle. When at rest the animal, as in a great number of planktonic organisms, is transparent. Striking changes in transparency accompany muscular activity, there being an early increase in transparency followed by a large increase in opacity.

Dr B. C. Abbott has collaborated with Prof. A. V. Hill and Mr J. V. Howarth of University College London on a basic investigation of heat production in isolated crab nerve.

The measurements of heat production during repetitive stimulation at room temperature indicated that an initial rapid outburst of heat was followed by an absorption of heat (so-called negative heat). The rapidity of the changes limited the precision of time analysis of the events; but it was possible to record the heat changes at 0° C following a single impulse in the nerve. The results confirmed the earlier results, showing an initial rapid positive phase followed by a longer lasting negative phase of heat. During such measurements the nerves have to conduct for several centimetres, and the wide range of propagation velocities complicates the time relationships. Records of the action potentials have been made in order to study the total times involved and the range of velocities; and also to ensure that the majority of the fibres are conducting.

In attempts to indicate the origin of the heat production it has been necessary to investigate the ionic exchanges occurring during nervous activity at 0° C. This is done using radioactive potassium which enables the fluxes to be measured: these experiments are still proceeding.

The light produced by fish-photophores is intermittent, and much remains obscure about the method of regulation. Utilizing material of a hardy luminescent species, *Porichthys*, which he collected while in California in 1954, Dr J. A. C. Nicol has investigated the possibility of nervous control. Histological studies have revealed that the photogenic tissue receives nerve fibres, and electrical stimulation of the nervous system (spinal cord) has been used to evoke the response. The pattern of innervation suggested that the light organs are supplied by the sympathetic system. An account of this work has been accepted for publication in the *Quarterly Journal of Microscopical Science*. An unusual bloom of *Noctiluca* in 1955 provided abundant material for physiological studies. Recordings were made of the luminescent flashes of single cells, and the effect of varying conditions of stimulation on the excitability of the cell studied. Cells luminesce only when stimulated (electrically and mechanically), when they give off brief flashes, some 0·1 sec. in duration. With repeated stimulation the light becomes weaker, but gradually recovers after a rest period. Several shocks produce a brighter response, owing to build up of some favouring agent. This work is to be continued when further material is available.

In collaboration with Dr G. Y. Kennedy of the Cancer Research Unit, Sheffield, Dr Nicol has been investigating the green pigments of *Chaetopterus*. These pigments are a mixture of phaeophorbides, localized in apparently symbiotic inclusions in the gut wall. An account of this work will soon be ready for publication.

Dr E. J. Denton has begun investigations on vision in marine fishes, both coastal and deep sea. Using an apparatus designed to make measurements of spectral absorption on very small areas of retina, he has worked on the photosensitive pigments found in the retinae of fish. In collaboration with Miss M. A. Walker of the Institute of Ophthalmology, London, the retinal rods of *Conger vulgaris* were shown to contain neither the rose-coloured 'visual purple' found in most coastal fish nor the purple-coloured 'visual violet' found in fresh water fish, but a golden coloured pigment. Dr Denton and Mr F. J. Warren adapted the apparatus for use at sea, and aboard R.V. *Sarsia* demonstrated similar golden coloured pigments in the retinae of four deep sea fish caught in the Bay of Biscay. The kindness of the Director of the National Institute of Oceanography enabled this result to be confirmed and extended aboard R.R.S. *Discovery II* to include in all fifteen species of deep sea fish. Alone amongst the oceanic fish studied, the surface fish *Saurus scombresox* was found to have a retina containing visual purple.

These golden coloured pigments, to which the names visual golds or chrysopsins have been given, have their maxima of absorption close to the maximum transmission of oceanic waters. The densities of photosensitive pigments found in the retinae of deep sea fish are very high; in some over 90% of light of wave-length 480 mμ is absorbed for one passage through the retina.

This figure may be compared with the corresponding figure of about 20% given for man by Dr W. A. H. Rushton. This is an adaptation to enable them to make most use of the feeble light found in the deep ocean.

THE LIBRARY

The thanks of the Association are again due to many foreign Government Departments, to Universities and to other Institutions at home and abroad for copies of books and current numbers of periodicals either presented to the Library or received in exchange for the *Journal* of the Association.

Thanks are also due to those who have sent books or reprints of their papers, which are much appreciated.

The Library has again been much used by visiting members of the Association.

The actual movement of the contents of the library, necessitated by the completion of the new extension and the re-classification of all material, was begun in May 1956. The Council wish to record their appreciation of the skill and devotion of the librarian, Miss L. M. Serpell, in completing the reorganization without interference to the normal use of the library.

PUBLISHED MEMOIRS

Volume 35, No. 2, of the *Journal* was published in June 1956, Volume 35, No. 3, in October 1956 and Volume 36, No. 1, in February 1957.

The following papers, the outcome of work done at the Plymouth laboratory, have been published elsewhere than in the *Journal* of the Association:

ABBOTT, B. C. & LOWY, J., 1955. Heat production in a smooth muscle. Proc. physiol. Soc., 23–24 September 1955. *J. Physiol.*, Vol. 130, 25P.

ABBOTT, B. C. & LOWY, J., 1956. A new muscle preparation for the study of optical changes during contraction. *Nature, Lond.*, Vol. 177, pp. 788–9.

ABBOTT, B. C. & LOWY, J., 1956. Resting tension in snail muscle. *Nature, Lond.*, Vol. 178, pp. 147–8.

ABBOTT, B. C. & LOWY, J., 1956. Early tension changes during contraction of certain invertebrate muscles. Proc. physiol. Soc., 2 June 1956. *J. Physiol.*, Vol. 133, 8–9P.

ABBOTT, B. C. & LOWY, J., 1957. Stress relaxation in muscle. *Proc. Roy. Soc.* B, Vol. 146, pp. 281–8.

ASHHURST, DOREEN E., 1956. Red blood-cell antigens in some lower vertebrates. *J. exp. Biol.*, Vol. 33, pp. 249–55.

ATKINS, D., 1956. Ciliary feeding mechanisms of brachiopods. *Nature, Lond.*, Vol. 177, pp. 706–7.

ATKINS, W. R. G. & JENKINS, PAMELA G., 1956. Factors affecting the vernal phytoplankton outburst in the English Channel. *Nature, Lond.*, Vol. 177, pp. 1218–19.

BALLANTINE, D. & ABBOTT, B. C., 1957. Toxic marine flagellates; their occurrence and physiological effects on animals. *J. gen. Microbiol.*, Vol. 16, pp. 274–81.

Carlisle, D. B., 1956. An indole-alkylamine regulating heart-beat in Crustacea. Proc. biochem. Soc., 25 May 1956. *Biochem. J.*, Vol. 63, 32P.

Carlisle, D. B., 1957. The direct action of anterior pituitary extracts on the initiation of lactation in the rabbit. *Physiol. Comp. Oecol.*, Vol. 4, pp. 295–312.

Clark, R. B., 1956. The eyes and the photonegative behaviour of *Nephtys* (Annelida, Polychaeta). *J. exp. Biol.*, Vol. 33, pp. 461–77.

Cooper, L. N. H., 1955. Deep water movements in the North Atlantic as a link between climatic changes around Iceland and biological productivity of the English Channel and Celtic Sea. *J. Mar. Res.*, Vol. 14, pp. 347–62.

Crisp, D. J. & Southward, A. J., 1956. Demonstration of small-scale water-currents by means of milk. *Nature, Lond.*, Vol. 178, p. 1076.

Denton, E. J., 1956. On the vision of the conger eel. Proc. physiol. Soc., 20–21 July 1956. *J. Physiol.*, Vol. 133, 56–7P.

Denton, E. J. & Warren, F. J., 1956. Visual pigments of deep-sea fish. *Nature, Lond.*, Vol. 178, p. 1059.

Hedley, R. H., 1956. Studies of serpulid tube formation. II. The calcium-secreting glands in the peristomium of *Spirorbis*, *Hydroides*, and *Serpula*. *Quart. J. micr. Sci.*, Vol. 97, pp. 421–7.

Hobson, G. E. & Rees, K. R., 1957. The annelid phosphokinases. *Biochem. J.*, Vol. 65, pp. 305–7.

Horridge, Adrian, 1956. The nervous system of Ephyra larva of *Aurellia aurita*. *Quart. J. micr. Sci.*, Vol. 97, pp. 59–74.

Jackman, L. A. J., 1957. *Marine Aquaria*, 137 pp. London: Cassell and Co. Ltd.

Jones, W. C., 1956. Colloidal properties of the mesogloea in species of *Leucosolenia*. *Quart. J. Micr. Sci.*, Vol. 97, pp. 269–85.

Keynes, R. D. & Lewis, P. R., 1956. The intracellular calcium contents of some invertebrate nerves. *J. Physiol.*, Vol. 134, pp. 399–407.

Knowles, Francis G. W. & Carlisle, David B., 1956. Endocrine control in the Crustacea. *Biol. Rev.*, Vol. 31, pp. 396–473.

Leston, D., 1956. Systematics of the marine-bug. *Nature, Lond.*, Vol. 176, pp. 427–8.

Millott, N. & Yoshida, M., 1956. Reactions to shading in the sea urchin, *Psammechinus miliaris* (Gmelin). *Nature, Lond.*, Vol. 178, p. 1300.

Murray, R. W., 1957. Evidence for a mechanoreceptive function of the ampullae of Lorenzini. *Nature, Lond.*, Vol. 179, pp. 106–7.

Oldfield, E., 1955. Observations on the anatomy and mode of life of *Lasaea rubra* (Montagu) and *Turtonia minuta* (Fabricius). *Proc. malac. Soc. Lond.*, Vol. 31, pp. 226–49.

Ross, D. M., 1957. Quick and slow contractions in the isolated sphincter of the sea anemone, *Calliactis parasitica*. *J. exp. Biol.*, Vol. 34, pp. 11–28.

Russell, F. S., 1956. Hydromedusae: Order: Anthomedusae. Families: Rathkeidae and Bougainvilliidae. (Zooplankton, Sheet **51**.) (1953.) Hydromedusae: Families: Pandeidae and Tiarannidae. (Zooplankton, Sheet **54**.) (1955.) *Fich. Ident. Zoopl.*, nos. 50–60.

Smith, J. E., 1957. The nervous anatomy of the body segments of nereid polychaetes. *Phil. Trans.*, B, Vol. 240, pp. 135–6.

Southward, A. J., 1956. The population balance between limpets and seaweeds on wave-beaten rocky shores. *Ann. Rep. for* 1955 (*no.* 68) *Mar. Biol. Stn Port Erin*, pp. 20–9.

Walker, Mary Ann, 1956. Homogeneity tests on visual pigment solutions from two sea fish. Proc. physiol. Soc., 20–21 July 1956. *J. Physiol.*, Vol. 133, 56P.

Welsh, J. H., 1955. On the nature and action of coelenterate toxins. *Pap. Mar. Biol. and Oceanogr., Deep-Sea Research*, suppl. to Vol. 3, pp. 287–97.

MEMBERSHIP OF THE ASSOCIATION

The total number of members on 31 March 1957 was 900, being 39 more than on 31 March 1956; of these the number of life members was 109 and of annual members 791. The number of associate members is four.

During the year Mr E. H. E. Havelock, C.B., C.B.E., Dr A. G. Huntsman and Dr A. C. Redfield have been elected Honorary Members.

FINANCE

General Fund. The thanks of the Council are again due to the Development Commissioners for their continued support of the general work of the laboratory.

Private Income The Council gratefully acknowledge the following generous grants received during the year:

From the Fishmongers' Company (£400), The Royal Society (£100) British Association (£50), Physiological Society (£50), the Cornwall Sea Fisheries Committee (£10), the Universities of London (£210), Cambridge (£125), Oxford (£100), Bristol (£50), Birmingham (£31. 10*s*.), Leeds (£20), Durham (£10. 10*s*.), Manchester (£10. 10*s*.), Sheffield (£10. 10*s*.), Southampton (£31. 10*s*.), Reading (£10. 10*s*.), Nottingham (£10. 10*s*.), Hull (£10. 10*s*.), Exeter (£10. 10*s*.), Leicester (£10. 10*s*.), the Imperial College of Science and Technology (£10), Gonville and Caius College, Cambridge (£5), and the Zoological Society of London (£10. 10*s*.).

President, Vice-Presidents, Officers and Council

The following is the list of those proposed by the Council for election for the year 1957–58:

President

Prof. A. V. Hill, C.H., O.B.E., Sc.D., LL.D., F.R.S.

Vice-Presidents

The Earl of Iveagh, K.G., C.B., C.M.G.
Sir Nicholas E. Waterhouse, K.B.E.
Col. Sir Edward T. Peel, K.B.E., D.S.O., M.C.
Vice-Admiral Sir John A. Edgell, K.B.E., C.B., F.R.S.
Sir Edward J. Salisbury, Kt., C.B.E., D.Sc., F.R.S.
Admiral Sir Aubrey C. H. Smith, K.B.E., C.B., M.V.O.
A. T. A. Dobson, C.B., C.V.O., C.B.E.
Major E. G. Christie-Miller
Morley H. Neale, C.B.E.
The Earl of Verulam
Prof. Sir James Gray, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S.

COUNCIL

To retire in 1958

Miss Vera Fretter, D.Sc.
N. A. Mackintosh, C.B.E., D.Sc.
G. E. Newell, Ph.D.
Prof. Lily Newton, D.Sc.
R. S. Wimpenny, O.B.E.

To retire in 1959

G. E. R. Deacon, C.B.E., D.Sc., F.R.S.
M. N. Hill, Ph.D.
O. D. Hunt
Prof. R. J. Pumphrey, Sc.D., F.R.S.
Prof. G. P. Wells, Sc.D., F.R.S.

To retire in 1960

Prof. E. Baldwin, Ph.D.
C. H. Mortimer, Dr.phil., D.Sc.
C. F. A. Pantin, Sc.D., F.R.S.
Prof. J. E. Smith, Sc.D.
H. G. Vevers, M.B.E., D.Phil.

Hon. Treasurer

Harrison S. Edwards, Westhumble Lacey, Near Dorking, Surrey

Secretary

F. S. Russell, C.B.E., D.S.C., D.F.C., F.R.S.
The Laboratory, Citadel Hill, Plymouth

The following Governors are also members of the Council:

R. G. R. Wall (Ministry of Agriculture, Fisheries and Food)
The Worshipful Company of Fishmongers:
  The Prime Warden
  Major E. G. Christie-Miller
  Harrison S. Edwards
Prof. A. C. Hardy, D.Sc., F.R.S. (Oxford University)
S. Smith, Ph.D. (Cambridge University)
Edward Hindle, Sc.D., F.R.S. (British Association)
N. B. Marshall (Zoological Society)
Prof. Sir James Gray, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S. (Royal Society)

BALANCE SHEET 1956–57

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

BALANCE SHEET 31 MARCH 1957

	£	£
CAPITAL RESERVE ACCOUNT:		
As at 31 March 1956	174,827	
Add: Expenditure on fixed assets recovered	1,257	
		176,084
SURPLUS ACCOUNT:		
As at 31 March 1956	8,856	
Add: Composition Fees fund transferred	1,089	
	9,945	
Less: Excess of expenditure over income for the year	3,163	
		6,782
		182,866
BALANCES ON SPECIAL FUNDS (see annexed statement)		6,902
CURRENT LIABILITIES:		
Sundry creditors and accrued expenses	4,055	
Subscriptions received in advance	46	
		4,101
		£193,869

Note: Capital commitments outstanding amount to £10,400 (1956 £10,193) of which £9,300 will be recoverable under Development Fund grants.

JOHN E. HARRIS }
N. B. MARSHALL } *Members of the Council*

	Cost, etc. £	Depreciation £	£
FIXED ASSETS:			
Boats and equipment:			
At cost:			
R.V. 'Sarsia'	137,761	—	137,761
At valuation as estimated by the Director at 31 March 1957:			
M.F.V. 'Sula'	12,500	—	12,500
R.L. 'Gammarus'	200	—	200
Laboratory apparatus, equipment and machinery:			
At cost *less* depreciation	14,637	2,668	11,969
Library at valuation by Mr Ridgill Trout in January 1941 plus additions as valued by the Director	22,300	—	22,300
	187,398	2,668	
			184,730
INVESTMENTS:			
General Fund (including Composition Fees) at book amount (Market value £1,277; last year £1,219)		1,609	
E. T. Browne Bequest Funds at cost (Market value £3,346; last year £3,180)		4,497	
		6,106	
Less: Provision for diminution in value of investments		1,483	
			4,623
CURRENT ASSETS:			
Stocks on hand as valued by the Director		1,700	
Sundry debtors		982	
Prepayments		276	
Balances at bank and cash in hand		1,558	
			4,516
			£193,869

AUDITORS' REPORT TO THE MEMBERS OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM:

Capital expenditure on the erection of buildings on land held on lease from the War Department is excluded. Subject to the foregoing, in our opinion the above balance sheet and annexed income and expenditure account give a true and fair view of the state of the Association's affairs as at 31 March 1957 and of its excess of expenditure over income for the year ended on that date.

We have obtained all the information and explanations which we considered necessary. In our opinion the Association has kept proper books and the said accounts which are in agreement with them and with the said information and explanations, give in the prescribed manner the information required by the Companies Act 1948.

Norwich Union House
2 St Andrew's Cross
Plymouth
24 *May* 1957

PRICE WATERHOUSE & CO.
Chartered Accountants

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 MARCH 1957

	£	£
Salaries, National Insurance and Superannuation Scheme Contributions		35,084
Laboratory and Boats' Crews' Wages, National Insurance, Superannuation Scheme Contributions and Employers' Liability Insurance		29,202
Upkeep of Library		709
Scientific Publications, *less* Sales		2,015
Upkeep of Laboratories:		
Buildings and machinery	910	
Electricity, gas, coal and water	1,254	
Chemicals and apparatus	1,965	
Depreciation of Laboratory apparatus, equipment and machinery (no depreciation is provided on the other fixed assets)	1,330	
Rents and insurance	219	
Travelling expenses	792	
Audit fee	126	
Stationery, postage, telephone and sundries	1,433	
Specimens	196	
Collecting expenses and upkeep of truck	359	
		8,584
Maintenance of Boats:		
Petrol, oil, paraffin, etc.	1,866	
Maintenance and repairs	6,371	
Insurances	2,303	
Hire of Decca Navigator—R.V. 'Sarsia'	395	
		10,935
Entertainment Expenses		39
		£86,568

	£	£	£
Grants and Table Rents:			
Ministry of Agriculture, Fisheries and Food Grant from Development Fund		74,172	
Fishmongers' Company		400	
Miscellaneous (including Royal Society £100, British Association £50, Physiological Society £50, Cornwall Sea Fisheries Committee £10, Universities of London £210, Cambridge £125, Oxford £100, Bristol £50, Birmingham £31. 10*s*., Leeds £20, Southampton £31. 10*s*., Durham £10. 10*s*., Exeter £10. 10*s*., Leicester £10. 10*s*., Manchester £10. 10*s*., Nottingham £10. 10*s*., Hull £10. 10*s*., Reading £10. 10*s*., and Sheffield £10. 10*s*., Imperial College, £10, Zoological Society of London £10. 10*s*., Ministry of Works £104, Imperial Chemical Industries Ltd. £52. 10*s*., International Paints Ltd. £52. 10*s*., Gonville and Caius College, Cambridge £5) ...		1,209	
			75,781
Subscriptions (excluding those received in advance)			802
Sales:			
Specimens		2,901	
Fish		1,015	
Nets, gear and hydrographical apparatus	850		
Less: Cost of materials	636		
		214	
			4,130
Sundry Publications			2
Income from Investments			53
Interest on Bank Deposits, *less* Charges			171
Aquarium:			
Admission Fees		2,561	
Sale of guides		54	
		2,615	
Less: Maintenance, printing and advertising		149	
			2,466
Balance being excess of expenditure over income for the year ...			3,163
			£86,568

MOVEMENTS ON SPECIAL FUNDS DURING THE YEAR TO 31 MARCH 1957

	Aquarium Sinking Fund £	Com-position Fees £	E. T. Browne Bequest			A. R. T. Momber Bequest £	Rockefeller Foundation Fund £	Library Extension and Dogfish House £	'Plymouth Marine Fauna' Fund £	Reservoir and Sea Water Tanks £	Research Funds* £	TOTAL £
			Library £	Special Apparatus £	Scientific Publications £							
BALANCE AT 31 MARCH 1956 (after providing £1,487 for diminution in value of investments)	386	1,030	1,012	2,114	325	1,124	(9)	1,353	(3)	—	190	7,522
Add: Income during year												
Grants	—	—	—	—	—	—	2,672	2,425	—	3,335	2,515	10,947
Income from investments	—	—	40	84	17	—	—	—	—	—	—	141
Bank deposit interest	13	—	—	—	—	39	—	—	—	—	—	52
Other income	32	48	—	—	121	—	—	—	—	—	—	201
Transfers between Special Funds	—	—	—	—	—	(1,163)	—	—	—	1,163	—	—
Decrease in provision for diminution in value of investments	—	11	12	24	1	—	—	—	—	—	—	48
	431	1,089	1,064	2,222	464	—	2,663	3,778	(3)	4,498	2,705	18,911
Deduct: Expenditure during year	—	—	13	170	—	—	2,394	2,591	—	3,335	2,417	10,920
Transfer to General Fund	—	1,089	—	—	—	—	—	—	—	—	—	1,089
BALANCE AT 31 MARCH 1957	£431	—	£1,051	£2,052	£464	—	£269	£1,187	£(3)	£1,163	£288	£6,902

* Including International Paints Ltd. Research Fellowship.